ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume XLVI, Art. 1. Pages 1-126

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1945, 1947

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ANIMAL COLONY MAINTENANCE

BY

Edmond J. Farris, F. G. Carnochan, C. N. W. Cumming, Sidney Farber, Carl G. Hartman, Frederick B. Hutt, J. K. Loosli, Clarence A. Mills, Herbert L. Ratcliffe

1817

NEW YORK
Published by the Academy
June 15, 1945

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VOLUME XLVI, ART. 1, PAGES 1–126
JUNE 15, 1945

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BY

EDMOND J. FARRIS, F. G. CARNOCHAN, C. N. W. CUMMING, SIDNEY FARBER, CARL G. HARTMAN, FREDERICK B. HUTT, J. K. LOOSLI, CLARENCE A. MILLS, AND HERBERT L. RATCLIFFE

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* This series of papers is the result of a conference on Animal Colony Maintenance held by the Section of Biology of The New York Academy of Science, November 10 and 11, 1944.

Publication made possible through a grant from the income of the Conference Publications Revolving Fund.

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INTRODUCTION TO THE CONFERENCE ON ANIMAL COLONY MAINTENANCE

By Edmond J. Farris

The Wistar Institute, Philadelphia, Pa.

It is indeed a pleasure to welcome you here on behalf of The New York Academy of Sciences, and it is my privilege to call this meeting to order. I wish particularly to express my appreciation and gratitude to the men who have so kindly contributed the formal papers for the program. The speakers are distinguished in their respective fields, and their messages will prove stimulating to this conference for frank discussion.

This conference results from the fact that there are many unsettled problems regarding maintenance of laboratory animals. I recall in my undergraduate student days in physiology (and I might add in my days of great inexperience with animals), after spending several hours preparing for and conducting a "type" mammalian experiment, my result hardly ever agreed with the usual textbook picture. This was common classroom experience. I inquired of the professor, "What's wrong?" The stock response was given, "If it's not your technique, it's the animal." As a graduate student, I had opportunity to observe some of the methods of maintaining laboratory animals, and my retort courteous to my professor, today, would be "Check the laboratory animal quarters. Check the animal caretaker and his methods. Know the animal's history and background." I am sure all the fine equipment on display in many laboratories will not aid in solving biological problems until satisfactory animals for experimental purposes are available.

As biologists, we are more or less consciously substituting a laboratory animal for man—in effort to solve problems presented by man.

Animal experimentation is usually justified on the grounds that the results may be carried over to man, who is an inconvenient experimental animal.

In the past, this was done in qualitative terms merely. For example, a given diet proved inadequate for the growth of a mammal, and we expected it would prove inadequate for man, too. Today, anatomical, physiological, and nutritional research has become quantitative in character. In any such investigations, a relatively uniform animal is desirable. A geneticist prefers mutations and variations, and rightfully so for his purpose. Yet, it is evident that healthy, clean, vigorous stock is essential for accurate research. A standardized animal is needed, for a standardized animal is to the biologist what the pure chemical is to the chemist.

Certainly, to secure such an animal is hardly possible, but, by proper precautions, a nearly standardized animal or group of animals for research purposes should be the aim of most laboratories.

As biologists, we know that the lower mammals differ from higher in being less able to regulate their physiological processes. They are more directly responsive to changes in environment. Respiration, pulse rate and temperature changes are examples of this lack of regulation. It is familiar to us, also, that appearance of a stranger in the colony disturbs animals, as evidenced at the dairy or chicken farm, where the output of milk and eggs is lowered. The animals, though long domesticated, are readily disturbed.

The animal is a very sensitive bit of apparatus. As the late Dr. Henry Donaldson once described the rat, it has more tricks than a string galvanometer, and must be treated with care and consideration. I recall that, on one occasion, simply moving rats from one building to another prevented breeding for several months.

The animal should be contented and happy, and this condition is not easy to attain, but is necessary.

The papers presented at this conference cover the problems involved in animal colony maintenance, and deal especially with six topics: namely, genetic purity; the mating of mammals; feeding laboratory animals; infectious diseases of laboratory animals; temperature control; and, finally, financing and budgeting.

GENETIC PURITY IN ANIMAL COLONIES

By F. B. Hutt

Department of Poultry Husbandry, Cornell University, Ithaca, N. Y.

INTRODUCTION

This symposium arises from recognition of the fact that, while aims and methods differ, there are some problems common to the maintenance of all experimental animals, whether the colony be a half-dozen cows in the feed-lot or a few hundred white rats in the wire-bottomed cages of the nutritionist. The guinea pig may be to the bacteriologist merely a test-tube, to the student of tick-borne diseases a culture medium, and to the geneticist a source of intriguing mutations; but all three can profitably compare notes on its maintenance, even though they view it through spectacles of different colors.

It seems desirable to state at the outset that this discussion of genetic purity and any suggestions made in this paper are not directed to the speaker's fellow-geneticists. This does not imply that there are no problems in their animal colonies or that they are particularly impervious to suggestions. It is merely that their conception of an animal colony is so different from that of other biologists.

The physiologist, the pathologist, and particularly the nutritionist want a steady supply of healthy animals with a minimum of variability. The anatomist appreciates variability a little more. He is accustomed to determining the range in size of an organ, not merely its mean. One hears frequently of the need for greater uniformity in the animals used for experiments in nutrition. The widespread utilization for this purpose of the inbred rats of the Wistar strain indicates the desire, not only to reduce variability to a minimum within any one experiment, but also to prevent as far as possible undue discrepancies between results at different institutions. When white rats of one strain gain only 14 grams in five weeks on a diet low in thiamine and those of another strain gain over twice as much on the same diet, the interpretation of results is likely to be confused unless the two strains are in one laboratory, and the difference, therefore, properly attributed to genetic differences between strains, as was done by Light and Cracas (1938)[1], rather than to variation in diets or in environment.

The geneticist, on the other hand, thrives on variability. It is his stock-in-trade. The recalcitrant rat that lingers on long after its orthodox litter-mates have terminated their abbreviated careers on the diet lacking vitamin Q is merely a statistical nuisance to the nutritionist, but, to the geneticist, it is the potential progenitor of a race able to manage nicely with much less vitamin Q than millions of rats that are less fortunate in the matter of genes. Sometimes, the geneticist may, by inbreeding, eliminate all innate variability except the two or three mutations currently under study, an accomplishment that is particularly easy if his animal colony consists of a few thousand *Drosophila* in pint milk bottles. In other cases, he may induce mutations by heat, by x-rays, or by other means, or he may accumulate naturally occurring mutations to make his various strains larger or smaller, blacker or whiter, more resistant or more susceptible to this or that disease. All of these things he is able to do without special advice or encouragement on this occasion, but he will be interested in what his fellow biologists can tell him about feeding, housing, management and other topics of this symposium.

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[1] **Light, R. F., & L. J. Cracas.** Science **87:** 90. 1938.

GENETIC DIFFERENCES BETWEEN INDIVIDUALS

It is a common belief that genetic variability is eliminated by using litter mates as experimental and control animals. While this is true of litter mates in a highly inbred line, it does not hold good for others. Litter mates may differ as much as any two siblings born several weeks, months or years apart. Because of this, and because mutations occur even in inbred lines, it seems desirable to show by a few examples how much a mutation in a single gene locus of the thousands that determine an animal's inheritance may affect its anatomy, physiology, or chances of survival. Because some biologists still think that genes cause only such "sports" as freaks of hair color, of hair form, of eye-color, or other inconsequential mutations, but have little or no effect on fundamental physiology, these examples are chosen to refute that view.

Consider the effects of the gene A^y, one of a series of multiple alleles affecting hair color in the house mouse. In the almost forty years that have elapsed since Cuenot reported that yellow mice do not breed true, it has been clearly shown that this gene is lethal to the homozygote, which dies *in utero*. The result is a ratio of 2 yellow (heterozygotes) to 1 non-yellow. The heterozygotes are characterized by (1) adiposity (Danforth, 1927),[2] (2) a sub-normal metabolism (Benedict and Lee, 1936),[3] and, as Castle (1941)[4] has shown, (3) a slightly greater body size, apart from their excessive fat. In addition to all these effects, or because of some of them, the yellow mice are (4) less susceptible to spontaneous mammary carcinoma than are their black or brown litter mates (Little, 1934).[5] It is probable that, with further study, still more peculiarities of physiology will eventually be added to the multiple effects of this gene that causes yellow hair.

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[2] **Danforth, C. H.** J. Hered. **18:** 153–162. 1927.
[3] **Benedict, F. G., & R. C. Lee.** Annales de Physiol. **12:** 983–1064. 1936.
[4] **Castle, W. E.** Genetics **26:** 177–191. 1941.

In the Frizzle fowl, the feathers curl back toward the head. Studies by Landauer and Dunn (1930)[6] and by Hutt (1930)[7] showed that this is caused by an autosomal dominant gene. When frizzled fowls of show-room type are mated together, they yield progeny that are so extremely frizzled as to appear woolly, others of standard type, and some not frizzled at all, the three types in a ratio of 1:2:1. The extremely frizzled birds eventually become more or less bare as their feathers break off. Landauer (1942)[8] and his co-workers have reported that these homozygous Frizzles differ from normal fowls in (1) viability, (2) rate of growth, (3) age of sexual maturity, (4) metabolism, (5) rate of heart beat and size of heart, (6) frequency of different types of blood cells, (7) size of endocrine glands, and still other ways. Similarly a gene for nakedness (Hutt and Sturkie, 1938)[9] is lethal to about half of the affected chicks before hatching, to half of those that do hatch in the first six weeks, and leaves the remainder with physiological handicaps similar to those of the homozygous Frizzles. There are at least three mutations that cause different degrees of nakedness in the mouse, and similar genes have been studied in rats, rabbits, cats, dogs and other animals.

A good example of a simple recessive mutation that prevents the functioning of an important endocrine gland is the dwarfism discovered by Snell (1929)[10] in the house mouse, and shown by Smith and MacDowell (1931)[11] to be caused by failure of the anterior lobe of the pituitary to secrete the growth-promoting hormone. This mutation is particularly interesting, because, while it inhibits that function of the anterior pituitary, it does not affect its secretion of the gonadotropic hormone. Manifold effects of the mutation, (as reviewed by Grüneberg, 1943)[12] include (1) endocrine malfunction, (2) an adult weight about one-quarter of normal, (3) complete sterility in both sexes, (4) histological abnormalities in the anterior lobe, (5) small and abnormal thyroids, (6) subnormal metabolism, (7) infantile structure of the thymus, adrenals and gonads, and (8) subnormal viability. In the rat, there is a simple recessive dwarfism which, though making mature dwarfs only half the size of their normal litter mates, resembles that in the mouse in causing complete sterility.

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[5] **Little, C. C.** J. Exper. Med. **59:** 229–250. 1934.
[6] **Landauer, W., & L. C. Dunn.** J. Hered. **21:** 290–305. 1930.
[7] **Hutt, F. B.** J. Genetics **23:** 109–127. 1930.
[8] **Landauer, W.** Biol. Symposia **6:** 127–166. 1942.
[9] **Hutt, F. B., & P. D. Sturkie.** J. Hered. **29:** 370–379. 1938.
[10] **Snell, G. D.** Proc. Nat. Acad. Sci. **15:** 733–734. 1929.
[11] **Smith, P. E., & E. C. MacDowell.** Anat. Rec. **46:** 249–257. 1931.
[12] **Grüneberg, H.** The Genetics of the Mouse. xii & 412 pp. Cambridge Univ. Press. 1943.

All dwarfism is not associated with endocrine malfunction and physiological abnormalities. The speaker is now studying a type of dwarfism in the fowl in which the affected birds apparently lay as many eggs and reproduce as well as their normal sisters, even though almost half the normal size. It is particularly interesting because it is sex-linked and recessive. This means that a male, heterozygous for the gene, will produce daughters half of which are normal and half dwarfed, even when he is mated with unrelated normal hens. The mutation was sent in for study by the breeder in whose flock it first appeared. He, like those who participate in this symposium, wished to maintain the genetic purity of his colony.

Particularly interesting and important to the man trying to maintain some uniformity in his animals, are the genes that cause some abnormality of metabolism. A good example is the inability of Dalmatian coach dogs to break uric acid down to allantoin as is done by most other dogs. As a result, they excrete over four times as much uric acid per kilogram of body weight per day as do other dogs. The difference, as Trimble and Keeler (1938)[13] have shown, is determined by a recessive mutation and is not linked with the spotting that characterizes the breed. Another genetic abnormality of protein metabolism causes the accumulation of the pigment, porphyrin, in the bones and teeth, its excretion in the urine and extreme photosensitivity. It is a recessive mutation in cattle (Fourie, 1939)[14] and apparently also in man. One mutation prevents the oxidation of phenylalanine beyond the stage of phenylpyruvic acid, and another causes the incomplete katabolism of tyrosine, with resultant excretion of homogentisic acid and the condition known as alcaptonuria. It seems probable that an abnormality in the metabolism of fats is responsible for the accumulation of sphingomyelin in the tissues of persons afflicted with amaurotic idiocy, a condition which is a simple recessive character. Although these last three examples of mutations affecting metabolism are so far known only in man, there is no reason why they should not be found in any other mammal.

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[13] **Trimble, H. C., & C. E. Keeler.** J. Hered. 29: 280–289. 1938.
[14] **Fourie, P. J. J.** Onderstepoort J. Vet. Sci. and Animal Ind. **13:** 383–398. 1939.

Resistance to disease is usually dependent upon more than one gene, but there is at least one case on record in which a simple recessive mutation was responsible for the loss of an entire strain of animals. This happened in the guinea pigs that were genetically unable to form blood complement (Rich, 1923).[15] These were demonstrated experimentally to be extremely susceptible to infection with *B. cholerae suis* and also proved so susceptible to natural infections that attempts to preserve them even by distribution in several different laboratories were of no avail.

Finally, no mutations have as disastrous effects as the lethal genes. In the homozygous condition, these genes may terminate life before birth, immediately after it, or at various stages up to maturity, or even in later life. Sometimes, their effects are so great that even the heterozygote is visibly affected, as in the Creeper fowl, Dexter cattle, and yellow mice. More often, the carrier of the gene is apparently fully normal and detectable only by a breeding test. Since these genes lessen the efficiency of reproduction, they are particularly important to all animal breeders. Apparently, they are abundant in all species. Ten years ago, a review of lethal mutations then known in domestic animals (Hutt, 1934)[16] listed only 31, but many more have been discovered in the intervening decade, and a recent check-list compiled by Lerner (1944)[17] includes 70 lethals in farm animals, with no less than 25 in cattle alone. Undoubtedly, more will be discovered. Lethal genes should cause little trouble in the strains of laboratory animals that are already highly inbred, but otherwise their presence is to be generally expected, and particularly so where small litters or high post-natal mortality prevail.

GENETIC DIFFERENCE BETWEEN BREEDS AND STRAINS

The foregoing examples were chosen to show that single gene substitutions (or deletions, which may be responsible for some lethal mutations) may have very great effects. Even more important, so far as physiological characters are concerned, are the multiple factors, each exerting a small effect, but in their cumulative action responsible for big differences in form and function. They are important to the breeder of live stock because they affect rate of growth, body size, the utilization of feed, the capacity to produce milk or eggs and other characters of economic value. They are important to the biologist for these same reasons, but also because they differentiate strains with respect to nutritional requirements, endocrine functions, resistance to disease, and other important characteristics, and, by so doing, cause discrepancies between experiments and disagreements between results in different laboratories.

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[15] **Rich, F. A.** Vermont Agric. Exper. Sta. Bull. **230:** 24 pp. 1923.
[16] **Hutt, F. B.** Cornell Vet. **24:** 1–25. 1934.
[17] **Lerner, I. M.** J. Hered. **35:** 219–224. 1944.

A few examples will illustrate the importance of these differences. Considering nutritional requirements, Light and Cracas (1938)[1] found significant differences in the rates of growth of three strains of rats on a diet deficient in thiamine. After pointing out that variations in the factor for the conversion of Sherman-Chase units to International Units could be explained by differences between strains, they pointed out that each laboratory should determine a conversion factor for its particular strain of rats. Similarly, White Leghorns require less thiamine than Rhode Island Reds (Lamoreux and Hutt, 1939).[18] On a diet containing 30 parts per million of manganese, Gallup and Norris (1939)[19] found White Leghorns to be completely free of abnormalities, while, in New Hampshires, 7 per cent of one strain and 18 per cent of another developed perosis, a condition indicating an unusually high requirement of manganese for bone development. Gowen (1936)[20] found that strains of rats differed greatly in their requirements of vitamin D, and white pigs, according to Johnson and Palmer (1939),[21] can store, during exposure to sunshine, a reserve of vitamin D sufficient to last about twice as long after confinement as the reserve stored by black pigs under the same conditions.

Apart from requirements of specific vitamins or minerals, Morris, Palmer and Kennedy (1933)[22] were able to establish by selection two strains of rats, one of which was 40 per cent less efficient than the other in the utilization of food. Most of the differentiation was accomplished with only six generations of selection.

Students of cancer have provided extensive evidence that strains of mice differ not only in susceptibility to a single type of tumor, whether spontaneous or induced, but also in susceptibility to different kinds of tumors. Strains of animals differ also in susceptibility to bacterial diseases. An important point demonstrated by Gowen (1933)[23] is that genetic resistance to disease is more likely to be specific than general. Thus, Schott's mice, bred for resistance to *S. aertrycke*, could easily withstand a dose of that organism that was fatal to all mice of the Sil and W. F. lines, but the latter were much more resistant to the virus of pseudorabies than were the Schott mice. Against the antigenic poison, ricin, the Sil strain was most resistant, the W. F. line most susceptible and the Schott mice intermediate.

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[18] **Lamoreux, W. F., & F. B. Hutt.** J. Agric. Res. **58:** 307–316. 1939.

[19] **Gallup, W. D., & L. C. Norris.** Poultry Sci. **18:** 76–82. 1939.

[20] **Gowen, J. W.** Genetics **21:** 1–23. 1936.

[21] **Johnson, D. W., & L. S. Palmer.** J. Agric. Res. **58:** 929–940. 1939.

[22] **Morris, H. P., L. S. Palmer, & C. Kennedy.** Minn. Agric. Exper. Sta., Tech. Bull. **92,** 56 pp. 1933.

[23] **Gowen, J. W.** Quart. Rev. Biol. **8:** 338–347. 1933.

THE REDUCTION OF GENETIC VARIABILITY

Having thus emphasized the extent to which a single mutation with great effects or many little mutations with cumulative effects may upset the efficient conduct of any experiment with animals, some reassurance is desirable. The fact that many laboratories are operating animal colonies successfully without too much genetic sabotage provides that assurance. The question is merely, "How can the genetic variability in my stock be reduced and kept at a minimum?"

Breeds, Varieties and Strains. Variability is lowest in a "pure line." To Johanssen a pure line was a group of organisms descended from a single ancestor, all identical in genotype and homozygous with respect to every pair of alleles. A clone, such as a patch of potatoes, all reproduced vegetatively from a single tuber, could also be considered a pure line, even though heterozygous in some genes. Such a degree of genetic purity, though readily attained in Johanssen's self-fertilized beans, is not likely to be established or maintained in many animal colonies. For one thing, while almost complete homozygosity can be attained by eight generations of self-fertilization, it takes over sixteen to accomplish the same result with brother x sister matings, which afford the most intense inbreeding that is possible in animals. For another, the occurrence of new mutations tends to increase heterozygosity in any stock, even one that may once have been highly inbred. It seems probable also that, in laboratories where most of the animals are wanted for other experiments, there may be somewhat more difficulty in maintaining the brother x sister matings which King (1919)[24] used so effectively to reduce variability within inbred families of the justly famous Wistar rats.

It is questionable, however, if such a high degree of genetic purity be necessary in most colonies and, as will be shown later, there are advantages in having some genetic variability in one's experimental animals, at least in some kinds of research. Among the domestic animals, there are at hand large colonies in which different degrees of homozygosity have already been established by the breeders, and from which experimental animals may be drawn with some assurance of consistent performance.

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[24] **King, H. D.** J. Exper. Zool. **29:** 71–111. 1919.

For example, the poultry breeder unconsciously recognizes three different degrees of homozygosity in the population of domestic fowls. Firstly, there are the breeds, usually identified by the names of the places in which they were developed, such as Leghorn, Rhode Island Red, Sussex, Minorca, etc. While these were differentiated primarily on the basis of conformation and color, they also differ in many important physiological traits of which the founders of the breeds were quite unaware (Hutt, 1941).[25] Secondly, there are the varieties within a breed, usually distinguished by different colors, but sometimes by other mutations. Finally, the breeder recognizes within a variety different strains, duly tagged with the name of the breeder who developed them, and believed, usually rightly, to differ in such important characteristics as the ability to lay eggs, to withstand disease, or to win blue ribbons at the Boston poultry show.

Each of these classes,—breed, variety and strain—represents a different degree of inbreeding, and consequently of genetic purity or homozygosity, that narrows down the range of variation. To illustrate,—few Leghorns exceed 5 lbs. in weight at maturity, all lay white-shelled eggs, and all are characterized by yellow shanks. This has resulted merely from the collection of certain genes that determine these characteristics and the exclusion from the breed of genes that make bantams, giants, brown shells or slaty shanks. It is the process of selection, but it is also a measure of inbreeding. Genetic variability is not reduced very much further by the distinguishing characteristic of the White Leghorns, a dominant gene, *I*, which inhibits the formation of melanin. However, once that color variety is set apart, an additional degree of inbreeding is assured, because selection of breeding-stock is limited to the white birds. Finally, the development of strains within a variety is usually carried out by selection within a single flock, and the inbreeding process is thus carried one step further.

Breeds, varieties, strains or races are available in most of the animals that have been long domesticated and in the laboratory animals that reproduce rapidly. They represent different degrees of inbreeding,—of genetic purity—already available for the laboratory worker. Although genetic variability is consistently reduced with each of these steps, much heterozygosity still remains. Thus, all attempts to establish an inbred strain of fowls by brother x sister matings have thus far failed because of the many lethal genes present in the heterozygous condition in the foundation stock. However, this method has been successfully used to establish cultures of *Drosophila* that are either "pure lines" or as close to it as one may go in animals. Among mammals, brother x sister matings for many generations have yielded the highly homozygous Wistar rats, the guinea pigs of the United States Department of Agriculture and the dilute brown mice developed by Little which have been so widely used for the study of neoplasms.

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[25] **Hutt, F. B.** Proc. Seventh Internat. Congr., Genet. (Edin. 1939): 156–157. 1941.

Further Inbreeding. The extent of inbreeding desirable will vary with the species and the type of experiment. Workers with *Drosophila* and with mice have little difficulty in maintaining strains that are completely homozygous, or nearly so, by continuous brother x sister matings. The same could probably be done with rats, rabbits and guinea pigs, provided that enough animals could be reared to prevent the colony from being closed out by lethal genes, that inevitably show their effects as inbreeding progresses. Such a risk would be minimized by starting with animals already somewhat inbred and there is greater chance of success if special care be taken to select breeding stock from large litters that show a minimum of undesirable defects. After the first generation, several different families must be maintained at least until some of them have weathered the first six generations. This is the most critical period, because it is that in which the greatest reduction of heterozygosity occurs (figure 1). The process of inbreeding

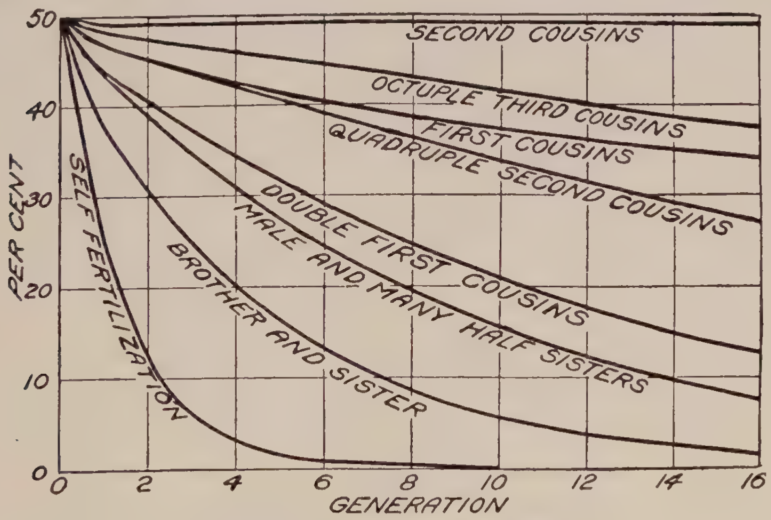

Figure 1. Rates at which heterozygosity is reduced with different degrees of inbreeding (From Wright, in U.S.D.A. Tech. Bull. 1121).

will be accompanied by differentiation into families, some of which will have undesirable traits necessitating their elimination, and the breeder must expect a considerable loss of stock before he can establish a strain that is satisfactory.

If it were not for the occurrence of new mutations, inbred lines like these could be kept homozygous in all genes. Even allowing for new mutations, the use of such stocks should greatly reduce variability in most experiments. After 25 generations of brother x sister matings in the Wistar rats, King (1919)[24] found the coefficients of variability in body weight at a given age to be only 10.9 and 8.5 per cent in the inbred males and females, compared with coefficients of 18.0 and 14.8 in controls. A similar reduction in variability with inbreeding is to be expected in almost any character, except those in which variation induced by the environment is much greater than that controlled by genes.

In species or strains in which brother x sister matings have been tried without success, it is probable that some satisfactory degree of homozygosity can be established with a less intense form of inbreeding. Half a dozen attempts to establish an inbred line of fowls by brother x sister matings failed, but at the Iowa Experiment Station a strain of highly inbred Leghorns was produced by using birds more distantly related. This takes a longer time, but lessens the risk of complete failure. Wright (1931)[26] calculated that, whereas the residual heterozygosity is reduced 19.1 per cent with each generation of brother x sister matings, it is reduced only 11 per cent if a single male can be mated in each generation with a large number of his half sisters. The rates at which this system of mating and others decrease the heterozygosity are shown in FIGURE 1.

Finally, some reduction of variability can be induced merely by breeding within one colony and not introducing any new blood. Contrary to an opinion commonly held, this does not result in rapid inbreeding, if the breeding stock be chosen at random and if several different sires are used in each generation. With such a system, according to Wright (1931),[26] the heterozygosity is reduced in each generation by approximately $\frac{1}{8\,N}$, where N is the number of males used, provided that the number of females is "unlimited." Although the use of 30 or 40 females per generation is hardly "unlimited," their number is relatively unimportant to the formula if they are far in excess of the number of males, as is usually the case. Thus, if 10 males be used per generation, the remaining heterozygosity is reduced only about 1.25 per cent, but if only three are used, the figure is about 4.2 per cent. Such a system is not likely to cause much trouble in any colony. It is one that has been used successfully in many.

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[26] **Wright, S.** Genetics **16:** 97–159. 1931.

SELECTION COMBINED WITH INBREEDING. It is assumed that, in any species and with any degree of inbreeding, constant selection will be maintained to eliminate sibships in which undesirable mutations appear or which are reduced in number by lethal genes operating at early stages of development. Selection may also be practised to head the strain in any direction particularly desired for some special type of work. The available evidence indicates that it should not be difficult to make any strain more resistant to some particular disease, or less so, if that were desired, to have it with an unusually high or low requirement of some vitamin, to make its members capable of living longer than their unselected fellows, or to modify its sensitivity to various environmental influences.

To some extent, this has already been done in the differentiation of breeds and varieties, which, as was earlier pointed out, represent varying degree of inbreeding and hence of uniformity. The fact that these may differ in important physiological traits does not imply conscious selection by the founders of the breeds, but, more likely, a natural selection by widely different environments in different parts of the world. Anyone doing biological research should take advantage of these breed differences, in so far as they may facilitate certain types of work, and beware of them if they are likely to confuse others. For example, in addition to the differences between Leghorns and heavier breeds already mentioned, there are important differences in susceptibility to pullorum disease, and to the nematode, *Ascaridia lineata,* in ability to withstand high temperature, in thickness of egg shell, in broodiness, and in response to hormones. Some of these, and possibly all, are quite independent of differences in body size. Similarly, as Casey *et al.* (1936)[27] have shown, breeds of rabbits differ significantly in the number and proportion of different kinds of blood cells.

RECOMMENDATIONS. To reduce variability, therefore, the biologist should (1) learn the range of variation to be found in the species with which he works, (2) select the most suitable breed or variety for his purpose, (3) should, if possible, obtain foundation stock of a strain or strains already differentiated and proven suitable for that purpose, and preferably somewhat inbred, and (4) maintain the colony without introducing new blood and with as much inbreeding as can be practised without endangering vigor and reproduction. As new mutations appear, it will be desirable to learn, either by direct experimentation or from friendly geneticists, how they are inherited (and, hence, how best eliminated if that be necessary) and also to what extent they may affect the work in progress. The example of the yellow mouse was used deliberately to show that even an apparently harmless simple mutation affecting hair color may have manifold far-reaching effects.

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[27] **Casey, A. E., P. D. Rosahn, C. K. Hu, & L. Pearce.** J. Exper. Med. **64:** 453–469. 1936.

VARIATION IN ANIMALS RECENTLY DOMESTICATED

In the short period of seventy-six years that has passed since Darwin first drew to the attention of biologists the great extent to which animals vary under domestication, that variation has undoubtedly been somewhat increased. To some extent, the very animal colonies under consideration in this symposium are responsible for the differentiation of races, varieties, or even breeds. There are over 200 breeds or varieties of the domestic fowl, in many colors and patterns, and varying in size from a 500-gram bantam to a Jersey Giant ten times as heavy. In striking contrast, among over 90 skins of *Gallus gallus* (which is generally accepted as the ancestor of all domestic fowls), taken in the wild from Lombok through the Dutch East Indies, through Burma and Indo-China to Hainan, and now available at the American Museum of Natural History in the Whitney and Rothschild collections, there is so little variation in size and color that the geneticist accustomed to the motley assortment in the domesticated population, but not to the subtle intricacies of taxonomy, finds it difficult to see the differences between the four sub-species of the Red Jungle Fowl that are now recognized by the "splitting" ornithologists.

Similarly, it is to be expected that any species recently domesticated will, at first, show remarkable uniformity in size, in color, and probably also in physiological traits. So it is with the golden hamster, *Cricetus auratus*, which is currently becoming popular as a laboratory animal. However, as more and larger colonies are established, mutations will be accumulated and races will be differentiated, as has already been done with mice, rabbits and other rodents. This is especially so if the recently domesticated species is one that appeals to the fanciers, as has the budgerigar, *Melopsittacus undulatus*, or if it produces mutations of great economic value, as have the mink and the fox.

The remarkable uniformity of a wild population does not mean genetic homozygosity except for the genes that have been subject to natural selection. Lethal genes carried in the heterozygous condition are sure to be present, and, for that reason, close inbreeding is a more risky procedure with a newly domesticated species than with one long domesticated and in which some degree of inbreeding may have already occurred.

GENES, ENVIRONMENT AND VARIABILITY

Variation is inevitable, even in a stock so highly inbred that it is almost a pure line. Whether it be the fruit flies that develop abnormal abdomens on moist food but not on dry ones, the turkeys that get pendulous crops in the hot climate at Davis, California, but not at Tomales, which is closer to the sea (Asmundson and Hinshaw, 1938),[28] or the rats that develop avitaminosis on one diet but not on another; these are only illustrations of the fact recognized by all geneticists that the phenotype is the end result of the interaction of the genotype with the environment. Sometimes, the environmental influence is greatest when it is quite unsuspected. It has recently been shown (Hutt, *et al.*, 1944)[29] that mortality in adult fowls from neoplasms, principally lymphomatosis, depends to a great extent upon the exposure to the causative agents during the first two weeks of life. The use of two brooder houses, apparently alike in most respects, may result in quite different mortality rates from this disease during a test period that doesn't begin until 21 weeks after the chicks have left those brooder houses. (TABLE 1.)

TABLE 1.
DEATHS FROM NEOPLASMS BETWEEN 160 AND 500 DAYS OF AGE, PER CENT.
DATA FOR THREE YEARS

Strain	Birds number	Chicks brooded for the first two weeks in:	
		House F.	House B.
C. Resistant	1604	14	5
K. Resistant	942	15	6
Susceptible	768	30	16

The fact that this difference was quite unsuspected during six years of selection did not prevent the differentiation of the resistant and susceptible strains, but it did make that process much slower than it would have been had it been recognized earlier that exposure is more severe in a brooder house 40 feet from adult fowls than in one 40 yards away.

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[28] **Asmundson, V. S., & W. R. Hinshaw.** Poultry Sci. **17:** 276–285. 1938.

[29] **Hutt, F. B., R. K. Cole, M. Ball, J. H. Bruckner, & R. F. Ball.** Poultry Sci. **23:** 396–404. 1944.

Finally, lest the impossible be expected, it should be emphasized that there are some genetic characters in which variability cannot be eliminated even in highly inbred stocks and in a constant environment. The mutation, *radius incompletus,* is a simple recessive variant of wing venation in *Drosophila funebris.* It is not influenced by the environment, but its penetrance, or the proportion of homozygotes that show the character, varies greatly in different lines so highly inbred as to constitute practically pure lines. Timofeeff-Ressovsky (1927)[30] found that, in one of these, 39 per cent of the homozygous recessives failed to show the character, although breeding tests proved that those not showing it were identical in genotype with those that did. In other lines, the penetrance was higher, the differences being attributable to the action of other genes in the different lines. Other cases of incomplete penetrance are known.

The white spotting of guinea pigs depends upon a pair of recessive genes with major effects. Many genes with minor effects apparently permit different degrees of spotting in different lines, but Wright (1936)[31] found that even in highly inbred isogenic strains, the amount of white could vary from a mere trace to 100 per cent white in strains in which the median grade was about 50 per cent. By far the larger proportion of this variability was attributed by him to "developmental accidents" not genetic in origin. Obviously, inbreeding can not eliminate all variation in all characters.

IN DEFENSE OF GENETIC VARIABILITY

There can be no doubt that, for most kinds of laboratory work, it is desirable to have animals in which genetic variability is reduced to the lowest possible minimum. The nutritionist assaying vitamin D in fish oils is not concerned with the genetic variations in the rate of ossification in the chicks he uses as test animals but with differences in the potencies of different oils. Similarly, the cancer specialist, studying the role of environment in the causation of that disease, wants a minimum of variability in his resistant and susceptible strains. However, these strains could not have been provided for his use unless there had been genes responsible for differences in susceptibility and resistance by the accumulation of which the highly resistant or susceptible strains were developed.

Differences between individuals and between strains may lead to further research to explain discrepancies, and consequently to more complete knowledge and broader applications than are likely when uniform results are obtained in the first experiment.

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[30] **Timofeeff-Ressovsky, N. W.** Genetics **12:** 128–198. 1927.
[31] **Wright, S.** Genetics **21:** 758–787. 1936.

For example, perosis, or slipped tendon, is a genetic and nutritional abnormality of growing chicks in which, because of faulty osteogenesis, the tibio-tarsal joint becomes enlarged and twisted so that the tendon from the gastrocnemius muscle frequently slips out of the intercondylar groove, thus causing the chick to be crippled. It was shown by Wilgus, Norris and Heuser (1937)[32] that this condition was prevented, in most cases, by adding manganese to the diet. However, it was later found (Gallup and Norris, 1939)[19] that, while 30 parts per million prevented perosis in all Leghorns and 50 p.p.m. did so in most New Hampshires, some chickens developed the abnormality even on diets containing twice the latter amount. These exceptions led to further research and the discovery that some birds need not only more manganese than their fellows for normal osteogenesis, but also more choline (Jukes, 1940)[33] and that all require biotin (Jukes and Bird, 1942).[34] Perosis is rare in Leghorns kept on normal diets. If the heavier breeds were not comparatively susceptible, the abnormality would not have received as much study as it has in the past 15 years. That research, in turn, might have stopped with the discovery that some birds need more manganese than others, were it not for few non-conformists whose special requirements included also an excess of choline.

Although genetic variability has undoubtedly been most useful in providing the variants from which superior strains of animals and plants have been bred by selection, it should not be difficult to find, in nearly any field of biology, cases like the example just given, in which a lack of genetic purity has led directly to a valuable extension of knowledge. In such cases, genetic variability is an unrecognized blessing in temporary disguise.

SUMMARY

Litter mates differing by a simple unifactorial mutation may be extremely different in form, in physiology and in viability. Multiple factors with important cumulative effects cause important differences between breeds, varieties and strains with respect to nutritional requirements, resistance to disease, and other important functions.

To reduce genetic variability, the laboratory worker is recommended to select from the existing breeds and strains those already proven suit-

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[32] **Wilgus, H. S., Jr., L. C. Norris, & G. F. Heuser.** J. Nutrit. **14:** 155–167. 1937.
[33] **Jukes, T. H.** J. Biol. Chem. **134:** 789–790. 1940.
[34] **Jukes, T. H., & F. H. Bird.** Proc. Soc. Exper. Biol. Med. **49:** 231–232. 1942.

able for his purpose, and to reduce heterozygosity still further by the most intense form of inbreeding that is compatible with vigor and good reproduction. Results to be expected with different degrees of inbreeding and with selection are discussed.

It is pointed out that variation is comparatively slight in animals recently domesticated, that some variability may persist even in highly homozygous lines, and that a little variation in animal colonies is sometimes responsible for the extension of knowledge.

DISCUSSION OF THE PAPER

Dr. L. S. Strong (*Yale University School of Medicine, New Haven, Conn.*):

First, I want to congratulate Doctor Hutt for his excellent paper, presented at this symposium.

On two points, I disagree with Doctor Hutt. I would hesitate to use all variable strains of all animals. The investigator is used to his own animals. In other words, we are beginning to report results of two heterozygous resultant quantitative variables to demonstrate results in the field of cancer research. Little variation is gotten in any of them. I am quite sure it will probably not take place in our generation. Almost every variation that I have is not qualitative, but is quantitative. Age is a more important variable with quantitative strains of animals and should be taken into consideration.

Dr. T. F. Zucker (*College of Physicians and Surgeons, Columbia University, New York, N. Y.*):

From what Doctor Hutt has stated and the answers he has given to the questions, it might be helpful to mention the work done with rats. I have been looking for a geneticist working with rats and Doctor Gowen and I would like to know where geneticists are now working with them.

Doctor Hutt has spoken about Doctor Palmer's work with rats. One feature of this was production by selection of a strain in which more production was maintained. During the course of our work (rats and resistance to rickets, etc.), both strains were lost and no animals retained. Our animals gradually died off. Both strains presented a kind of problem which must be discussed.

An important quantitative problem is body size. Doctor Mendel pointed out stature as genetic and rate of growth as due to nutrition. In most cases, you produce mice by selection. Body weight and intrinsic weight are extreme. Food requirement is regulated by body weight. There is extreme variability in the rat. Doctor King has done important work on body weight by use of the coefficients of variability in body weight at given ages of the animals and Doctor Smith's work with inbred strains should be mentioned.

In our work at Columbia with the rat, the weight remained constant in all different types, as soon as we began selection for body size. In about three generations, we raised the weight from 45 to 60 in males and from a little over 50 to somewhat under 70. I wonder whether there is more information available on the various features in the genetics of the rat?

Reply by Dr. Hutt:

Till recently, most geneticists working with rats were more concerned with those variations brought about by genes which affect color, etc., than with the physiological characters, but studies of the latter are now being made. Some selection has been made in respect to requirements of vitamin D.

Dr. Myron Gordon (*American Museum of Natural History, New York, N. Y.*):

The National Research Council maintains a Committee of which Doctor Landauer is Chairman and includes Doctor Little of Bar Harbor. It is responsible for information concerning poultry. I am in charge of fishes.

I want to point out that some sort of committee should be established which will be able to supply information concerning the availability and location of various pure strains of animals needed for laboratory experimentation.

Capt. Roy Nichols (*Army Medical and Veterinary Schools, Washington, D. C.*):

It is not possible to call animals truly genetically identical in the genetic sense, until we have overcome the selection problem in the different laboratories and have standardized the strain genetically. Cannot each laboratory standardize its strains? Inevitably, when one strain has been kept genetically identical for several generations by selection and their location is changed, variation will occur in the animals.

Reply by Dr. Hutt:

A difference in the environment in different laboratories could bring about a difference in the strains. If one laboratory maintains its thermostat at 68° and another one at 80°, it would not be surprising if after several years the strains differed in their temperature requirements.

THE MATING OF MAMMALS

By Carl G. Hartman

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DEFINITION

The term, mating, may be used transitively or intransitively. In the latter sense, mating is usually used synonymously with copulation. More rarely, and more or less anthropomorphically, where the association of male and female is for a season or "for life" (foxes?, swans?, elephants?), the word, mating, is applied to this more permanent relation. The term in this sense begins to have social significance, and sex to become a factor in the organization and stability of the family.

In a state of nature, in herds on the range or large mixed colonies of laboratory mammals, the animals are free to choose their mates. But breeders or laboratory experimenters usually wish to control the mating of their animals. Hence, mating is employed in the sense of placing together males and females which are at other times isolated. In this transitive sense, the word is used synonymously with "breeding" or "hand mating," terms employed by breeders of the larger farm animals.

The present paper is concerned with such controlled matings as are carried out for exactitude in experimental work, or to increase fecundity by insuring fertilization of the ova.

The controlled breeding of animals by man antedates recorded history. It is only recently that it has been gradually emerging from the empirical to the scientific and becoming a science rather than an art. This advance is due to the great strides made, chiefly since the turn of the century, in the study of the physiology of reproduction. Since the mating techniques are based on scientific principles, some of the principles will be outlined before individual species are considered. After that, the common laboratory species will be discussed, together with some of the larger animals of agricultural importance.

RUT

Rut, or "heat," or estrus, is the limited period in which the female accepts the male. Every species has its own characteristic behavioral manifestations of estrus (see Young, 1941,[1] for a summary of these). Thus, the cow in heat will mount other cows; the rat will dart, vibrate her ears, and exhibit lordosis when approached by other rats. In all instances, the female in heat becomes very active and restless, and her food intake is greatly lessened. Teleologically speaking, this activity is favorable for the female to seek out the male.

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[1] **Young, W. C.** Quart. Rev. Biol. **16:** 135–156, 311–335. 1941.

In a broad sense, heat is closely related to ovulation, except in pathological cases, as, for example, in nymphomaniac cows. This holds rather strictly for the lower mammals, but is less apparent in monkeys and apes, in which we see the dawn of the social significance of sex. In man, finally, the picture is almost totally obscured, for a rise in sex desire is seldom reported as occurring in the middle of the cycle when ovulation certainly takes place (Ball and Hartman, 1935[2]; Hartman, 1936[3]).

THE PHYSIOLOGICAL BASIS OF ESTRUS

It has been known from time immemorial that removal of the gonads results in almost complete blotting out of the sexual response in the male (eunuchs, geldings, steers, capons). After the advent of abdominal surgery, the same was found true of the female also. It remained for the modern endocrinologist and the behaviorist to analyze the internal chemical milieu necessary to sensitize the nervous system so that the adequate stimulus (presence of the mate or sex partner) may elicit the appropriate response—i. e., "male" behavior of the male in the presence of the female, "female" behavior of the female in the presence of the male. It has been shown, however, in appropriate sex hormone experiments, that the chromosomal constitution (if that conditions sex behavior) may be overridden by the appropriate heterosexual hormone (Stone, 1939[4]; Ball, 1937,[24] 1940[20]; Beach, 1942[4a]). Certain forms of behavior, as mounting by a female in heat (cow, guinea pig, rat), have been interpreted as "male"; therefore, for the female, "homosexual" behavior. But this seems absurd, since it is a quite common form of behavior of females when in heat, in fact, is advisedly regarded by Young (1941)[1] as a helpful indicator of heat in the guinea pig, as it is also in the cow. The matter is of more than academic interest to the rabbit breeder, in that such mounting behavior of an estrous female often results in one or the other or both partners ovulating and becoming pseudopregnant and therefore sterile for the succeeding 16 days.

Sex behavior in the male is normally brought about by the male hormones, testosterone and androsterone. In the female, estrone and estradiol, secreted by the ovarian follicles, condition female sex behavior. In the intact female, as estrus approaches, her sex behavior becomes more and more pronounced, in correlation with the growth of the graafian follicle, and the most intense manifestation synchronizes with the maximal distension of the follicle. "Silent" estrus, that is, morphological changes leading to ovulation without overt behavioral manifestation, also occurs.

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[2] **Ball, Josephine, & C. G. Hartman.** Am. J. Obstet. and Gynec. **29:** 117–119. 1935.

[3] **Hartman, Carl G.** Time of Ovulation in Woman. Williams & Wilkins Co. Baltimore. 1936.

[4] **Stone, Calvin B.** Chapter on Sex and Internal Secretions. Edgar Allen, Ed. Baltimore. 1932 (1st Ed). 1939 (2nd Ed.).

[4a] **Beach, F. A.** Psychosomatic Med. **4:** 173–198. 1942.

In the castrate, estrogens alone are able to call forth mating behavior. Indeed, I have seen an ovariectomized bitch kept in heat constantly for several years. In the castrated rat and guinea pig, however, not all treated females will come into heat, but the refractory individuals, after being primed with estrogens, can be brought promptly into heat by a dose of progesterone. Furthermore, progesterone, added to estrogen, greatly intensifies the response. It seems very probable that, in the normal cycle, the ripe graafian follicle produces progesterone, which precipitates the copulatory response (and ovulation!—Everett, 1944),[5] for there is collateral evidence of progesterone production by the unruptured follicle (see review article by W. C. Young, 1941).[1]

Upon the establishment of the corpus luteum and the initiation of the "progesterone" phase of the cycle, the female becomes not only indifferent to the male but, in some species, positively antagonistic.[5a] In the latter case, the sexes should be separated after mating, at least not kept together in close quarters, for the female is likely to injure the male. I have seen a small opossum female, during the night after her heat period, kill a male double her weight.

To summarize: estrus in the lower mammals is limited to a few hours or a few days of the sexual cycle. The "cycle" may comprise a whole year in "monoestrous" animals—those having a single rutting season—such as deer, many wild rodents, and probably all marine mammals. The dog comes into heat twice, the cat perhaps six times in a year, the long period between estri being known as the "anoestrum" (Heape), in contrast to the "diestrum," the interval between short, regularly recurring estri of "polyoestrous" mammals. Some mammals—as, forexample, monkeys—experience cycles the year round, but are fertile only in a portion of the year (Hartman, 1931;[6] see also Zuckerman, 1931[7]). In some of our highly domesticated mammals (rat, guinea pig, rabbit, cow), the non-breeding season has all but been eliminated; less so in the horse, and still less so in the sheep, goat and ferret; the last three still experiencing rather marked though variable anestrous periods.

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[5] **Everett, J. W.** Endocrin. **34:** 136–137. 1944.

[5a] For the pathological human counterpart, see **Benedek, Th., & B. B. Rubenstein** Psychosomat. Med., Mono. **III.** 1942.

[6] **Hartman, Carl G.** J. Morph. **12:** 129–140. 1931.

[7] **Zuckerman, S.** Proc. Zool. Soc. London. **25B:** 343. 1931.

THE TIME OF OVULATION

In animal breeding, it is of the utmost practical importance to know exactly when the egg or eggs leave the ovary. It is, of course, true that multiple matings tend to insure fecundity to the maximum; but with expensive animals like stallions and bulls or even rams and boars, the animal husbandman must economize by making his males serve as many females as possible. The embryologist or the physiologist in the laboratory, less concerned about the cost of maintaining a few extra males of the laboratory rodents, finds great advantage in the precision with which the hour of ovulation may now be predicted in several species. Once the hour or even the day of ovulation is known, the female may be served at the most fertile moment. The necessity for precision in this matter arises out of the low period of viability of sperms in the female genital tract (about 30 hours) and the still more critical period of fertilizability of the ovum (about 10–15 hours), as is now generally recognized (Hartman, 1924,[8] 1932,[9] 1939;[10] Blandau and Young, 1939;[11] Blandau and Jordan, 1941[12]).

Knowledge of the time of ovulation with reference to outward signs —in the lower mammals, the beginning and end of estrus, in the primates, menstruation—is gradually being built up. Very accurate data, as will be seen below, exist for rat, mouse, guinea pig, rabbit, ferret, and cow, less accurate for sheep, goat and mare.

THE ENDOCRINOLOGY OF OVULATION

Categorically stated, the growth of the graafian follicle is brought about by the follicle stimulating hormone (F.S.H.) of the anterior pituitary, the rupture of the follicle by the addition of the luteinizing hormone (L.H.). It is, therefore, possible to precipitate ovulation and thus fix the time of ovulation with certainty. The classic example is the estrous rabbit, which will ovulate about 10 hours after injection of any of the ordinary gonadotrophs (Friedman test for pregnancy). The estrous cat and certain hibernating bats are further examples. Mirskaia and Petropavlosvsky (1937)[13] have taken advantage of the technique to precipitate ovulation in the estrous mare and hence to be certain of ovulation 30–48 hours after the treatment (1000 M. U. of human chorionic hormone).

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[8] **Hartman, Carl G.** Am. J. Obst. & Gynec. **7:** 40–43. 1924.
[9] **Hartman, Carl G.** Contributions to Embryology **13:** 1–162. 1932.
[10] **Hartman, Carl G.** Chapter on Sex and Internal Secretions. Edgar Allen, Ed. Baltimore. 1939.
[11] **Blandau, R. J., & W. C. Young.** Am. J. Anat. **46:** 303–329. 1939.
[12] **Blandau, R. J., & E. S. Jordan.** Am. J. Anat. **68:** 275–291. 1941.

For reasons which the writer has stated elsewhere (1942), attempts to (a) cure sterility, (b) increase litter size, and (c) interpolate an additional pregnancy in the anestrous period of animals by means of the administration of gonadotrophic hormones are still in the experimental stages. It is true that astounding results have been achieved in spots; but the overall fecundity of a flock or herd or colony has not been increased and the expense involved has been great.

The chief loss in cases of induced ovulation is due to the failure of the treated females to mate. "Silent" heat (ovulation without overt signs of estrus) has been reported for numerous species and may be expected in all. The phenomenon is most likely to occur at the beginning and at the end of the breeding season. "Silent" heat has been discovered in horses and cows in the course of routine palpation of the ovaries; in sheep, guinea pigs and rats, in the course of autopsies. Ovulation without estrus represents ova wasted, and there is nothing the animal breeder can do about it at present.

SPONTANEOUS VS. NERVE-INDUCED OVULATION

It has been known for a hundred years (ever since Barry and Bischoff in the 1840's) that the rabbit normally ovulates only after copulation, although, as late as 1908, the subject formed an amusing controversy between Ancel and Villemin, on the one hand, Dubreuil and Regaud, on the other (C. R. Soc. Biol. Paris, Vol. LXV), in which the former did the arguing, the latter the experimenting. Villemin even asks whether one is to believe that a rabbit female would ovulate by being ogled by a male in a nearby cage!

The mechanism by which nerve impulses are set up by the act of copulation is now clear in its broad outlines (see review in Allen, 1939[14]). Through nerve channels, via the hypothalamus and the hypophyseal stalk, impulses from the erogenous zones reach the anterior pituitary and there cause the outpouring of the ovulatory hormones. As a result, ovulation occurs some time later (within about 10 hours in the rabbit, 35 in the ferret).

In the class with the rabbit, which does not ovulate spontaneously, are the cat, the ferret and the marten; and to these O. P. Pearson (1944)[15] has added the remarkable case of the short-tailed shrew, in which the female requires the stimulus of a half dozen copulations daily for several days to consummate ovulation.

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[13] **Mirskaia, L. M., & V. V. Petropavlovsky.** Prob. An. Husb.: 22–39. 1937. (Abst. in Am. Breed. Abstr. **5**, Dec. 1937.)

[14] **Allen, Edgar.** Sex and Internal Secretions: 636–641. Baltimore. 1939.

ARTIFICIAL INSEMINATION

Under the leadership of Soviet scientists, the techniques of artificial insemination were brought to a high degree of efficiency and are now widely used in all countries. Males are conditioned to deposit their semen in artificial vaginae and this is then diluted, so that a single ejaculation serves to inseminate a variable number of females, depending on the species. By this method, for example, one ram, in a single season, is said to have sired over 2000 lambs.

This topic hardly comes under the subject under discussion. However, it should be noted that the males are conditioned to "mate" with dummies, and soon do so in preference to mating with females in heat.

Artificial insemination has a place not only in animal husbandry, but also for experimental purposes in the laboratory (cf. Blandau and Young,[11] Blandau and Jordan[12]).

CHANGING THE DIURNAL CYCLE BY MEANS OF LIGHT

Nocturnal animals mate at an hour of the night which greatly inconveniences the laboratory worker. It has been shown by Hemmingsen and Krarup (1937)[16] that, by turning night into day and day into night by means of electric lighting, the time when the majority of females come into heat may be changed to suit the experimenter. This is now very generally practised. It is recommended that the colony be illuminated from 6:00 p.m. to 6:00 a.m., and be darkened from 6:00 a.m. to 6:00 p.m.

VARIABILITY OF PHYSIOLOGICAL EVENTS

What is "normal" in physiological processes? Perhaps variability is the normal thing. If one examines large numbers of data, such as those of Long and Evans (1922)[17] on the rat, Bacsich and Wyburn (1940)[18] on the guinea pig, Andrews and McKenzie (1941)[19] on the

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[15] **Pearson, O. P.** Am. J. Anat. **75:** 39–93. 1944.

[16] **Hemmingsen, A. M., & N. B. Krarup.** Kgl. Danske Vidensk. Selskab. Biol. Med. **13:** 1–61. 1937.

[17] **Long, J. R., & H. M. Evans.** Mem. Univ. Calif. **6:** 1–148. 1922.

[18] **Bacsich, P., & C. M. Wyburn.** Proc. Roy. Soc. Edinb. **60** (Pt. I): 33–39. 1940.

[19] **Andrews, F. N., & F. F. McKenzie.** Res. Bull. **329,** Agric. Exp. Sta. Univ. of Mo. May. 1941.

horse, one is struck with the wide variations in both the total length of the estrous cycle of animals and in the segments of the cycle. Yet, within wide limits, judged by reproductive performance, variations are "normal."

It follows from this that, in fixing mating time, one must allow for some variation; for example, in the time of ovulation with reference to some other event of the cycle. This variability will detract somewhat from the precision of our mating precedures, but this is inevitable.

TECHNIQUES OF THE ANIMAL BREEDER

In the following summary, some details concerning special techniques in the control of timed matings will be discussed, but only where either exact data on the time of conception is desired, as for securing developmental stages of the embryo, or where economic considerations make it desirable or even imperative that insemination should result in pregnancy in a higher and higher percentage of cases, as in the breeding of high-bred horses and cattle.

Much animal breeding is done still, as always, by simply allowing the sexes to mingle at will and mate when a given female is in heat. As in the past, sheep and goats, horses and cattle are still self-propagated, as it were, on the range, where there are still wide open spaces and where man-power is scarce. In many laboratories, animals are reared in mixed colonies when numbers of adult animals are needed without regard to genetic constitution or exact age of the individuals. Thus, guinea pigs seem to thrive in sizable groups in pens, on the floor in space set aside for them in a convenient corner. Mice, rats, hamsters, kept—males and females together—in large cages, leave the experimenters the maximum of offspring with a minimum of effort.

The following remarks will be restricted to some of the principles which may serve as guides in the "hand breeding" of the various species. For symptoms or overt signs of heat in these, the reader is referred to the comprehensive review of W. C. Young (1941).[1]

THE RAT

Ovulation in the rat is related, temporally, with several easily determinable signs, namely, the vaginal smear, the voluntary or "spontaneous" activity, and the sexual response or estrus. A less exact procedure is by daily examination of females caged with males for the vaginal plug or the coagulated semen of the male.

The last method is sufficiently exact for most purposes, when it is recalled that most females come into heat between 6:00 p.m. and 3:00 a.m., with the peak round 11:00 p.m. If one institutes a regular 12-hour artificial day by electric illumination, one can work out for his own colony a curve of hour of ovulation and make his calculations on the basis of finding vaginal plugs early in the morning. Occasionally, a plug will fall out, giving a false negative, which, however, may be avoided, if one takes a vaginal smear in suspicious cases, as, for example, where one finds a swollen vulva and no plug.

In this connection, the novice, in the handling of a rat colony (the same holds also for mouse and probably hamster colonies), should have called to his attention, first, that copulation and ejaculation are not synonymous, for the rat may copulate many times without ejaculating. Second, at least two plugs should be allowed before the male is removed from the cage, for Ball (1940)[20] found that one-plug matings frequently afforded insufficient stimulus to the pituitary to activate the corpora lutea for progesterone secretion, indispensable for pregnancy changes in the uterus.

The original data of Long and Evans (1922)[17] on the relation of the vaginal smear picture to the time of ovulation have served as a good guide for many years. A female rat mated when the smear consists of large epithelial cells only, or with the addition of a few scales (early cornified stage), is likely to become pregnant. Young, Boling and Blandau (1941)[21] have reinvestigated this schedule and find that, by the time cornification is completed, the female rat has already ovulated.

Taking advantage of the voluntary running activity cycle of the female rat (Wang, 1923)[22] Farris (1942)[23] has determined quite precisely that sexual receptivity follows soon after her running begins to increase markedly and that ovulation occurs a little over 10 hours later in the non-mated, 8 hours later in mated individuals. Females mated while the activity is on the increase become pregnant in 9 out of 10 cases. The number of females that can be bred according to this schedule by this method is, of course, limited by the number of individual activity cages at one's disposal.

More accurate timing of ovulation and fertilization of the ovum is afforded by the method of determining the exact beginning of behavioral estrus. One does not need a male to make the test for

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[20] **Ball, Josephine.** Am. J. Physiol. **130:** 471–474. 1940.
[21] **Young, W. C., J. L. Boling, & R. J. Blandau.** Anat. Rec. **80:** 37–45. 1941.
[22] **Wang, G. H.** Comp. Psych. Monogr. **2:** 1–27. 1923.
[23] **Farris, E. J.** Anat. Rec. **84:** 4. 1942.

Ball (1937)[24] showed that the estrous female responds to the "finger test." This consists of quick, rhythmic clasping of the female just in front of the iliac crests, whereupon the rat, if she is in estrus, responds with lordosis, as when clasped by the male. Blandau, Boling and Young (1941)[25] have described and illustrated a modification of the test, which is applicable to the guinea pig and the hamster also.

Ovulation occurs about 10 hours after the onset of heat (Boling, Blandau, Soderwall and Young, 1941).[26]

In the work with the rat little advantage has been taken of the post-parturitional ovulation characteristic of many rodents. According to Blandau, Jordan and Soderwall (1940,)[27] the average interval between parturition and the beginning of heat was 18.5 hours (range 4–36) and that the temporal relation between the beginning of heat and ovulation was the same as in nonpregnant rats.

THE MOUSE

Certain facts, set forth above for the rat, hold also for the mouse. Ovulation occurs at a time when the vaginal contents include types of cells corresponding to those in the rat (Allen, 1922,[28] Snell *et al.*, 1940).[29] The latter authors report ovulation occurring in their mice shortly after midnight and not over 2 to 3 hours after the beginning of heat.

With those data in mind, to secure timed material, the breeder would do well routinely to look for vaginal plugs in the morning and to calculate the most probable hour of fertilization according to Snell and co-workers. It should be mentioned, however, that these authors used artificial "day and night" so as to control the rhythm of the mice more accurately; however, Lewis and Wright were able to secure many mouse ova in cleavage, without special illumination of the colony, by looking for vaginal plugs each morning in females caged with males.

Sobotta (1895)[30] secured nearly 1500 mouse ova by utilizing the postpartum ovulation, but he made no generalization as to the exact time when ovulation occurred. Long and Mark (1911)[31] studied this point carefully and reported that, in their mice, ovulation occurred 14½ to 28½ hours after delivery of the young.

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[24] **Ball, Josephine.** J. Comp. Psych. **24:** 135–144. 1937.

[25] **Blandau, R. J., J. L. Boling, & W. C. Young.** Anat. Rec. **79:** 453–463. 1941.

[26] **Boling, J. L., R. J. Blandau, A. L. Soderwall, & W. C. Young.** Anat. Rec. **79:** 313–331. 1941.

[27] **Blandau, R. J., E. S. Jordon, & A. L. Soderwall.** Anat. Rec. **78:** 58. 1940.

[28] **Allen, Edgar.** Am. J. Anat. **30:** 297–371. 1922.

[29] **Snell, G. D., E. FeKete, K. P. Hummel, & L. W. Law.** Anat. Rec. **76:** 39–54. 1940.

[30] **Sobotta, J.** Arch. f. Mikr. Anat. **45:** 15. 1895.

[31] **Long, J. R., & E. L. Mark.** Carnegie Inst. Wash. Publ. No. **142.** 1911.

Because of the smallness of the mice, they may be bred in numbers in relatively small cages, litters being removed and labeled as they appear.

THE GUINEA PIG

Nearly a century ago (1852), Bischoff[31a] and, after him, Hensen (1876)[31b] and Rein (1883)[31c] utilized the postpartum ovulation in order to mate their guinea pig females to secure "timed" embryological material. The method is still useful.

Working with the guinea pig, Stockard and Papanicolaou (1917)[32] gave us the "vaginal smear" method by which estrus and ovulation could be timed with a fair degree of accuracy. It has since been shown, however, by Young and co-workers (Young, 1937)[33] that, as in the rat, the time of ovulation may be predicted with a high decree of accuracy, by noting the time of beginning of estrus and calculating 10 hours forward. By the time the fully cornified stage is reached by the vaginal mucosa, most of the females are already out of heat. The rat and the guinea pig are alike in ovulating about 10 hours after the beginning of heat. The rabbit ovulates about 10 hours after mating.

THE RABBIT

As already stated, the rabbit belongs to that group of mammals which do not ovulate spontaneously, but only in response to a stimulus—either the nervous stimulus to the pituitary as a concomitant of copulation, or in response to exogenous gonadotrophic hormones (cf. cat, ferret, mink and marten).

During the breeding season—or at least that season in which rabbits breed best (cf. Marshall, 1922;[34] Hammond, 1925;[35] Hammond and Walton, 1934[35a])—it had been supposed that the doe remained in estrus ("constant estrus") for the season, in consequence of the presence of large follicles in the ovaries, and, hence, that she could be mated at any moment. A single crop of follicles was supposed to remain intact for the season. Neither of these conclusions is correct. In the first place, even a casual experience with a rabbit colony teaches that does pass through short periods when they will not take the buck. In the second place, it is now known that follicles come and go in cycles of about 15 or 16 days, one set degenerating while another set comes on (Smelser, Walton and Whetham, 1934).[36] It is probably in the transitional period, while the new set of follicles is growing and the old set is retrogressing, that the doe lacks interest in the male.

[31a] **Bischoff, Th. L. W.** Ent.-gesch. d. Meerschweincheneies, Giessen. 1852.
[31b] **Hensen, V.** Zeitschr. f. Anat. u. Entwickl.-gesch: 213, 351. 1876.
[31c] **Rein, G.** Arch. f. mikr. Anat. **22:** 233. 1883.
[32] **Stockard, C. R., & G. Papanicolaou.** Am. J. Anat. **22:** 225–283. 1917.
[33] **Young, W. C.** Anat. Rec. **67:** 305–325. 1937.
[34] **Marshall, F. H. A.** The Physiology of Reproduction, London. 1922.
[35] **Hammond, J.** Reproduction in the Rabbit. London. 1925.
[35a] **Hammond, J., & A. Walton.** J. Exp. Biol. **11:** 307–319. 1934.

Because of the sensitivity of the ovulatory mechanisms, it happens that, as stated above, females may stimulate each other to the point of ovulation! Does should, therefore, be kept isolated if "estrus" individuals are desired. Advantage may also be taken of the postpartum ovulation in the rabbit (Weil, 1873).[37] The postpartum doe constitutes, indeed, the most reliable stage for the Friedman pregnancy test. The government bulletins by Templeton (1940)[37a] and Templeton, Ashbrook and Kellogg (1942)[37b] will prove of practical use to every rabbit breeder.

THE GOLDEN HAMSTER

Too little is known about this species, which is destined to become an important laboratory rodent. The estrous cycle is about 4 days in length. We have seen hamsters mate in the early evening but have not studied them in detail. They are as easy to raise as rats. We have followed the practice of leaving one male with several females and isolating a female as soon as she is palpably pregnant. The hamster, like the rat, should be handled gently with frequency to keep it tame and tractable.

THE OPOSSUM

The vaginal smear of the opossum follows the general picture of the rat and the guinea pig in that the cornified stage is associated temporally with ovulation (Hartman, 1923).[38] There seems to be a considerable "postoestrous" period (between estrus and ovulation) not yet determined with any accuracy.

Before the advent of the vaginal smear technique, the writer was able to determine the approach of estrus in the opossum by the increasing swelling (edema) of the mammary glands (Hartman, 1921).[39]

Timed matings are best made in either of two ways: the less accurate one of examining the vaginal lavage mornings and evenings for sperms; or, more accurately, keeping a group of females with a number of males running free in a large dimly illuminated room under constant observation for cases of copulation. These occurred mostly at night.

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[36] **Smelser, G. K., A. Walton, & E. O. Whetham.** J. Exp. Biol. **11:** 352–363. 1934.
[37] **Weil.** Wiener Med. Jahrb. 1873.
[37a] **Templeton, G. S.** Wildlife Circular. U. S. Fish and Wildlife Service. 31 pp.
[37b] **Templeton, G. S., F. G. Ashbrook, & C. L. Kellogg.** Conserv. Bull. **25,** U. S. Fish and Wildlife Service. 63 pp.
[38] **Hartman, Carl G.** Am. J. Anat. **32:** 353–421. 1923.
[39] **Hartman, Carl G.** Am. J. Physiol. **55:** 308–309. 1921.

THE DOG

The dog family offers several exceptional features. The males do not possess seminal vesicles. In copulation, the male "hangs" or remains interlocked with the female. The act, in common with that of most carnivores, is long, upward of 20 minutes, which is quite the antithesis of the rabbit, the goat and indeed most mammals.

Ejaculation in the dog may be divided into three stages, as determined in part by masturbating the male, in part by observation through uterine fistula (Evans, 1933).[40] First, there is a clear secretion (from Cowper's gland?) almost devoid of sperms; second, a milky secretion containing clouds of sperms, perhaps the bulk of those furnished at a given mating; third, for most of the duration of coitus, a thin secretion of the prostate, carrying sperms in relatively small numbers.

The dog ovum (as also that of the fox) is peculiar in that the first maturation division does not take place until after it reaches the oviduct. There are, furthermore, some breeding data which point to a longer period of viability for the dog ovum than that of other mammals.

It seems anomalous that, for man's oldest domesticated animal, we cannot yet state definitely when ovulation occurs with reference (a) to the beginning of sexual receptivity, and (b) to the uterine bleeding. Evans and Cole (1931),[41] in their monograph on reproduction in the dog, state definitely that the bitch ovulates "usually within a day after the first acceptance of coitus." The autopsies recorded bear out this conclusion. However, practical dog breeder F. L. Whitney (1927,[42] 1942,[43]) who has bred thousands of dogs, places the "mode" for ovulation day on the 5th day of estrus. Whitney (1942)[43] also approached the problem by double matings with males of different breeds, one early in estrus, the other later. Matings on days 1 and 2 were sterile, later matings (days 5, 6 and 7) were fertile, as judged by the phenotype of the offspring.

Artificial insemination, with or without the use of gonadotrophic hormones, has not often been resorted to in the case of the dog.

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[40] **Evans, Everett.** Am. J. Physiol. **105:** 287. 1933.
[41] **Evans, H. M., & H. H. Cole.** Mem. Univ. Cal. **9:** 57–101. 1931.
[42] **Whitney, Leon F.** Chase Magazine ——. 1927.
[43] **Whitney, Leon F.** Vet. Med. **35**(3); See also **35**(1): 59–60. 1940.

THE CAT

The domestic cat displays marked symptoms of estrus, as described by Bard (1939, 1940),[44] and others quoted by Young (1941).[1] The overt signs of heat constitute sufficient guide for the investigator to regulate timed matings. Ovulation in the cat requires an extraneous stimulus, such as: artificial stimulation of the cervix (Greulich, 1934),[45] injection of gonadotrophic hormones (Windle, 1939)[46] or, normally, copulation. Eggs are discharged from the ovary about 30 hours later—in 26–27 hours according to Gros (1936),[47] in 25 hours after mechanical stimulation of the cervix, according to Greulich and Dawson and Friedgood.

THE FOX

Because of the recent expansion of the silver fox breeding industry, the U. S. Fish and Wildlife Service has had some intensive studies made on reproduction in this form, chiefly by O. P. Pearson and R. K. Enders of Swarthmore College,[48, 49, 50, 51] in collaboration with C. F. Bassett,[52] of the U. S. Fur Animal Experiment Station at Saratoga Springs (Bassett & Leekley 1942;[52a] Bassett, Wilke & Pearson, 1943[52b]).

The data presented by Pearson and Enders indicate that vixen tend to ovulate on the second day of receptivity. As is the case in all of the carnivores so far studied, estrus is slow in coming on. This is reflected in the gradually increased swelling of the vulva, the most reliable outward sign. As the breeding season approaches, vixen should be examined every third day for increased swelling of the vulva. When this approaches the maximal, the female should be tested with a reliable male for acceptance, and insemination should be allowed on the day following the first acceptance.

Heretofore, because most fox males are monogamous, testing vixen for acceptance has been difficult, since polygamous males, suitable for the test, have been scarce. The writer has the impression that, in recent years, the polygamous tendency has been bred into stocks, so that more such highly desirable males are now available.

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[44] **Bard, P.** Res. Publ. Assoc. Nerv. & Mental Dis. **19:** 190–218. 1939; **20:** 551–579. 1940.

[45] **Greulich, W. W.** Anat. Rec. **58:** 217–224. 1934.

[46] **Windle, W. F.** Endocrin. **25:** 365–371. 1939.

[47] **Gros, G.** Thèse Alger. 146 pp. 1936.

[48] **Pearson, O. P., & R. K. Enders.** Anat. Rec. **85:** 69–83. 1943.

[49] **Pearson, O. P., & R. K. Enders.** Am. Fur Breeder **17** (Jan.): 32–34. 1944(a).

[50] **Pearson, O. P., & R. K. Enders.** Am. Fur Breeder **17:** (July): 24, 26, 28; (Aug.): 26, 28, 30, 42. 1944(b).

[51] **Pearson, O. P., & R. K. Enders.** J. Exp. Zool. **95:** 21–35. 1944(c).

[52] **Pearson, O. P., & C. F. Bassett.** Anat. Rec. **89:** 455–459. 1944.

[52a] **Bassett, C. F., & J. R. Leekley.** N. A. Veter. **23:** 454–457. 1942.

[52b] **Bassett, C. F., F. Wilke, & O. P. Pearson.** Am. Fur Breeder **16:** 22–26. 1943; Black Fox Mag. **27:** 12, 13, 20, 21, 23, 25–27, 29. 1943.

FERRET, MINK AND MARTEN

Because of the value of the fur, the demand for it, and the growing scarcity of the feral population of our fur bearers, "fur farming" is steadily expanding. New mutations in mink are constantly appearing, which helps to enliven the mink-breeding industry. Marten breeding is just emerging from the experimental stage, and fisher and stoat have not been studied enough to be included in this discussion.

The Mustelidae mentioned, have, together with the most domesticated of them all, the ferret, several features in common: (1) they all require the stimulus of coitus for ovulation (Marshall, 1904,[53] 1922;[34] Pearson and Enders, Jan. 1944[49]); (2) the swelling of the vulva and dilatation of the introitus, which constitute, as in the fox, excellent signs of approaching estrus; (3) the act of copulation lasts unusually long, as in the dog.

The vaginal smear, though instructive, is less useful in the members of this family, than in the case of other mammals.

Studies on the physiology of reproduction in the mustelids have been made under the auspices of the U. S. Fish and Wildlife Service and others are in progress.

The ferret may be mated according to the vulvar enlargement. This attains, at estrus, a 50-fold increase over the anestrous condition. There is no "best" time for mating, as in spontaneously ovulating species—any time that the female accepts the male is satisfactory for breeding purposes. Ovulation occurs 30 hours, more or less, after mating. This entails the formation of the corpus luteum, in response to which the vulvar swelling recedes and pregnancy ensues—or, in case the ova are not fertilized, pseudopregnancy follows, a phenomenon which is quite marked in the carnivores.

Mink and marten are housed and bred much like the ferret (Leekley & Enders, 1941).[53a]

THE PIG

The average farmer who raises pigs for the market usually allows his sows and boars to run together and mate in their due season, nature "taking its course." The breeder of pure stock, however, must keep record of sire and dam and he therefore hand-breeds them.

From the behavior of sows, such as general activity (Altmann, 1941),[54] swelling of the vulva, behavior towards other sows (Corner, 1921),[55] the onset of estrus may be roughly diagnosed, and the animals mated accordingly. Heat lasts about a day. Mating at any part of estrus seems equally effective—which greatly simplifies the matter, as in the sheep, but in contrast with the cow and the mare.

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[53] **Marshall, F. H. A.** Quart. J. Micr. Sci. **48:** 323–345. 1904.
[53a] **Leekley, J. R., & R. K. Enders.** Am. Fur Breeder **14:** 26–28. 1941.
[54] **Altmann, Margaret.** J. Comp. Psych. **31:** 481–498. 1941.
[55] **Corner, G. W.** Contrib. to Embryol. **13:** 117–146. 1921.

Young boars (yearlings) should not be allowed more than a single mating a day, older boars, two, three, or up to four.

THE SHEEP

Most sheep are raised on the range and breed as wild animals do, rams and ewes, juveniles and lambs all ranging together. To guide the scientific breeder, however, extensive studies have been made on reproduction of both ewe and ram: breeding season, estrous cycle, time of ovulation, breeding capacity of rams, etc. For a comprehensive survey of the literature, as well as the most detailed observations, we have the monographs of McKenzie and Terrill (1937)[56] on the ewe, and McKenzie and Berliner (1937)[57] on the ram.

There are no very definite and reliable signs of estrus in the absence of the male. Hence, reliable rams are used for testing and records kept from actual observation of the mating. Estrus lasts around a day and a half on the average, with a wide spread for variations, and the cycle is about 16 days in length. In 88 per cent of 79 cases in which the time of ovulation in relation to the beginning of estrus was accurately determined by McKenzie and Terrill, this "preovulatory" period was found to be between 24 and 36 hours.

As ovulation in the ewe occurs near the end of heat, and since the heat period is not of long duration, a single service or single artificial insemination, whenever the ewe is in heat, is likely to result in pregnancy.

Ram semen is extremely concentrated as to sperm population and admits of great dilution for purposes of artificial insemination. Thirty million sperms are used at one insemination.

There are numerous reports of artificially stimulated ovulation of ewes in the non-breeding season; but it has not yet been established that this procedure is of commercial value. (cf. Casida and Murphree, 1942).[58]

THE COW

The estrous cycle of both cow and mare is stated to be around 21 days in length. The duration of estrus is short, about 16 hours; in Zebu cattle of West Africa (Anderson, 1936),[59] less than two hours.

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[56] **McKenzie, F. F., & C. E. Terrill.** Res. Bull. **264,** Agr. Exp. Sta. Univ. of Mo. (July), 88 pp. 1937.

[57] **McKenzie, F. F., & V. Berliner.** Res. Bull. **265,** Agr. Exp. Station, Univ. of Mo. 1937.

[58] **Casida, L. E., & R. L. Murphree.** Endocrin. **31:** 545–548. 1942.

The cow discloses its estrous condition by marked behavior, well known to every farm boy: mounting other cows, restlessness and running, bellowing. Taking advantage of these signs and having herdsmen report the first appearance on 200 cows, Mirskaia, of the Soviet Institute of Artificial Insemination near Moscow, in 1935,[60] removed the ovaries from these animals at different periods after the onset of heat and determined from an examination of these ovaries the average time of ovulation. This proved to be one of about 36 hours. This agrees very well with the findings of Nalbandov and Casida (1942),[61] who place ovulation about 22 hours after the end of heat; and, since estrus is about 16 hours in length, we get 38 hours as the pre-ovulatory period.

One would be inclined to mate or artificially inseminate a cow as soon as she is found in heat, unless one knows the exact time of onset, in which case it would be best to wait 10–12 hours.

THE MARE

The diestrous cycle of the mare is generally given as 20–21 days, which is a good average, but there is a large spread in the curve of cycle lengths. Estrus lasts most often 3–5 days, but is sometimes shorter, more often longer (see table pp. 8 and 9 of Andrews and McKenzie, 1941).[19] Estrus is best determined by the use of a "teaser" stallion.

Ovulation in the mare has been determined with precision by rectal palpation of the ovaries (Mirskaia 1935;[60] Hammond, 1938b).[61] This procedure was, of course, for purposes of research only, as it is not a practical measure for the breeder. The time of ovulation may also be inferred on the basis of timed matings (Hammond, 1938a).[62] The Soviet workers, as well as Hammond of England, and Andrews and McKenzie are agreed that ovulation occurs 24–48 hours before the end of estrus.

There is little consolation in this conclusion for the horse breeder, however, for the preovulatory period is very variable (due, according to Hammond, to the variability of the different rates of growth of follicle in different mares). It may be stated, on the basis of known facts, that it is better not to breed the mare at the beginning of the heat period.

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[59] **Anderson, James.** Emp. J. Exper. Agric. **4:** 186–195. 1936.
[60] **Mirskaia, L. M.** Sechenov J. Physiol. U. S. S. R. **21:** 195–196. 1935.
[61] **Nalbandov, A., & F. C. Casida.** J. An. Sci. **1:** 189–198. 1942.
[62] **Hammond, J.** Yorkshire Agric. Soc. J. **95:** 11–25. 1938(a).

Mirskaia and Petropavlovski (1937)[13] circumvented the dilemma by precipitating ovulation by means of human chorionic hormone. One thousand mouse units injected on the third or fourth day of heat may be expected to cause ovulation within 24 or 36 hours. Artificial insemination or mating would be indicated late on the same day, or better, on the day following the injection.

THE RHESUS MONKEY

From among the macaques indigenous to southeastern Asia, the rhesus monkey, *Macaca mulatta,* is the one which has established itself among us as a laboratory animal. I doubt whether the popularity of this species is due to superior qualities over others. It is probably its availability that is responsible. The animal is, however, hardy, long-lived and fertile in captivity.

For the taxonomic position and the wide geographical distribution of the races of the species, the reader is referred to the volume, "Anatomy of the Rhesus Monkey," Hartman and Straus, Ed., Williams and Wilkins, Baltimore, 1933. In the appendix of this volume will be found a description of various monkey quarters, including that of the Carnegie Laboratory of Embryology in Baltimore. Methods of handling monkeys, when kept in large paddocks, are also discussed.

As large males are dangerous to handle, it has been my practice to catch them only when they need to be transferred from one cage to another, treated for an injury, or the like. Instead, for breeding purposes, it is the females that are brought to the males. Appropriate sliding doors are provided for separating the two animals in order to remove the female.

Preparatory to mating, it is well to let the female become accustomed both to the cage and to the male or males to which she is to be bred; for, once she is frightened, she is likely to be attacked by the male and injured, which renders mating subsequently even more precarious. This difficulty is greatly lessened when only a few monkeys are confined in a room where they can see and "talk" to each other. Under such circumstances, Dr. Gertrude van Wagenen, at Yale University, has been very successful in breeding monkeys and rearing the young to maturity.

As I first found about 1930, it is possible to palpate the ovaries of the monkey and thereby determine with accuracy the time of ovulation (Hartman, 1930[62a]). Many hundreds of observations have been made, from which I selected 337, concerning which there was no element of doubt as to the time of ovulation (Hartman, 1932,[9] 1936,[3] 1944[63]). In these, ovulation occurred 149 times on day 12 or 13, and 273 times between day 11 and day 14.

[62a] **Hartman, Carl G.** Anat. Rec. **45:** 263. 1930.

Around the middle of the cycle is, therefore, the optimum time to mate the monkey, if a pregnancy is desired.

The chimpanzee has a 36-day menstrual cycle, as compared with one of 28 days characteristic for the macaque female and for women (Hartman, 1936,[3] 1944[63]); and ovulates correspondingly later, i.e., around days 17–20 (See Yerkes, Chimpanzees, Yale University Press 1943, for references).

DISCUSSION OF THE PAPER

Dr. Robert K. Enders (*Swarthmore College, Swarthmore, Pa.*):

How fitting it is that this paper should be read by a man who has had such wide experience in mating mammals maintained in laboratory colonies, and that it should be read in a museum which was among the first to realize the need and develop the facilities for such colonies.

The wide taxonomic range that has been covered emphasizes the richness of the fauna that can be used in the laboratory. The work of Dr. Hartman has benefited by his knowledge of which species of mammals to use for a given research. It is only fair to assume that even greater riches will be uncovered as we become more familiar with a greater number of species. Thus, to mention but two new additions to the laboratory colony, the hamster and the cotton rat have opened fresh approaches to old fields of investigation. The discovery of still other mammals that can live under laboratory conditions is only a question of imagination and trial.

Dr. Hartman has spoken of the techniques of the animal breeder. The importance of technique is often overlooked and may result in failure or in data of doubtful value. In a statistical study of the breeding of mink, Apelgren (1941)[1] came to the conclusion that, as far as productivity was concerned, not more than 10 per cent was due to heredity, the remaining 90 per cent being due to external causes. Work by O. P. Pearson on the mink and fox indicates that at least 50 per cent of the variation in litter size is due to factors not genetic. These figures are based on large numbers of animals kept on different ranches and are mentioned here because they emphasize the need for just such information as has been given on mating, and they also emphasize the importance of technique in caring for animals.

While many breeders believe that the tendency to polygamous breeding by male foxes is inherited, I rather incline to the belief that this is training. If a male fox is permitted to spend the winter and the early part of the breeding season with one female, it may be difficult to induce him to breed with a strange vixen. On the other hand, if the dog fox has not been so treated, skillful handling will lead to willingness to mate with almost any estrous vixen.

There are few exceptions to the generalization that the use of hormones has not led to any increase in the overall fecundity of a herd or colony. The use of hormones in securing more pregnancies in the horse has been mentioned, and to this should be added the findings of Dryerre,[2] who reported that the production of a herd of foxes was increased by injecting prolan and thus bringing about, not greater production in the individual vixen, but an increase in the percentage of yearling vixens bred.

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[63] **Hartman, Carl G.** West J. Surg., Obstet. & Gynec. (July) **52:** 41–61. 1944.

[1] **Apelgren, Rune.** Kullstorleken och dräktighetstidens längd hos mink. Våra Pälsdjur. **12:** 349–351, 6 figs. 1941.

[2] **Dryerre, H.** Prolan and fertility in silver fox. J. Physiol. **96:** 35P–36P. 1939.

Dr. Frank A. Beach (*American Museum of Natural History, New York, N. Y.*):

Dr. Hartman quite properly stresses the specificity of the behavioral effects of ovarian and testicular hormones; but, if our thinking is to remain clear, we must be careful to avoid overemphasis upon this point. When administration of androgen induces masculine behavior in a genetic female, or when estrogen causes feminine responses in a male, how are we to interpret the phenomena? Dr. Hartman suggests that the heterologous hormone overrides the chromosomal contitution, and this view is entirely sufficient for practical purposes, but I prefer to think of the situation in somewhat different terms. Experimental data now available strongly suggest that the normal chromosomal constitution of the genetic male includes provision for the mediation of feminine sexual behavior; and a similar bisexual arrangement appears to exist in the genetic female. Administration of the heterologous hormone merely increases the reactivity of those mechanisms responsible for the overt responses typical of the opposite sex. The organization of the homologous mechanism is not affected, although its responsiveness to stimulation may be greatly reduced.

Along this same line it may be noted that the homologous or, perhaps we had better say, the *prepotent* behavioral mechanism may, under certain conditions, be activated by the heterosexual hormone. Thus, it is not strictly accurate to say that "estrogens alone are able to call forth mating behavior in the castrate female"; for the spayed female rat may be induced to receive the male, if she has been injected with androgen. Furthermore, the castrated male rat exhibits increased masculine sexual performance when injected with estrogen. It is of interest in this connection to note the reports of some clinicians to the effect that libido in the human female may be increased by testosterone propionate treatment more consistently than by ovarian hormone administration.

In speaking of the difficulties of increasing fertility by induced ovulation, Dr. Hartman mentioned the high incidence of "silent heat"—ovulation which is not accompanied by willingness to receive the male—and he observed that, "there is nothing that the animal breeder can do about it." I should like to add that the experimentalist interested in behavior should be able to do a great deal about it, and thus make a valuable contribution. Some headway in this direction has already been made in studies revealing different thresholds for the morphological and behavioral aspects of estrus. There is no reason why similar experimentation on a larger scale should not provide us with means of simultaneously inducing sexual receptivity and ovulation in domesticated species.

Dr. Hartman observed that male animals may be conditioned to mate with dummies in preference to a receptive female; and I am inclined to feel that this point deserves special attention because of its implications for practical animal breeding. It is well known that potentially valuable sires may be ruined for stud by improper handling during early services; and, conversely, the readiness with which a male attempts to mate with a female may be increased by appropriate conditioning in early adulthood. It has been repeatedly demonstrated that a male's sexual responsiveness can be either raised or lowered by previous experience, and regular schedules for the sexual conditioning of animals to be used at stud seem worthy of consideration.

One final point that may be mentioned rests upon the common observation that fertility, potency and libido are three separate functions which behave somewhat as independent variables. Thus a male or female may be fertile and yet fail to reproduce because of absence of sexual drive. Again, it is possible that a fertile male may never impregnate a female because of his impotence. Proper hormone treatment makes it possible, not only to increase fertility in the sense of inducing ovulation or spermatogenesis, but also to intensify the sex drive and thus induce or increase overt mating responses. Sexually sluggish but completely fertile males often mate readily and effectively following the administration of small doses of androgen—doses which are not damaging to the seminiferous tubules. Similarly, females showing socalled silent heat may be induced to receive the male and to conceive, if appropriate hormone administration is employed.

Dr. Edmond J. Farris (*The Wistar Institute, Philadelphia, Pa.*):

Dr. Hartman mentioned in the course of his paper that I should describe some of our findings on the study of reproduction in the albino rat. In our studies on reproduction in the female albino rat, a method was developed to establish, by running activity, puberty, estrus cycle regularity, four stages of estrus and menopause. With the aid of a blackboard, these findings can be quickly demonstrated. During estrus, in stage I, the female fought the male, and no intercourse occurred. In stage II of estrus (two hours after beginning of heat, as determined by the start of the females' running activity), 90 per cent of the resistant female rats became pregnant when permitted to mate no more than twenty minutes. The male ejaculated once at between the 15th to 18th minute of this twenty minute mating period. In stage III, both the male and the female mated willingly, and only 10 per cent pregnancies occurred. It required usually 55 to 80 minutes of mating before the male would ejaculate, during this stage. In stage IV, the male was uninterested in the female in heat.

It was established that ovulation occurred in Wistar albino rats eight hours after the beginning of heat in mated females, and slightly later in the non-mated.

Spermatozoa were identified in the uterus promptly after ejaculation. However, spermatozoa were seldom found in the oviduct or ovarian sac until just before or at time of ovulation.

Finally, the spermatozoa became immobile in the uterus four hours after ovulation, and disappeared completely from the uterus within three days after ovulation.

Dr. Arthur Zitrin and Dr. Frank A. Beach (*Department of Animal Behavior, American Museum of Natural History, New York, N. Y.*)[1]:

In the discussion of Dr. Hartman's paper on the mating of mammals, one of us mentioned the fact that we have been able to "condition" male cats so that they will mate frequently and consistently under laboratory conditions. The incidental statement called forth queries emphasizing the rather widely accepted belief that the domestic cat does not readily reproduce in the laboratory. This attitude is reflected in the reports of Winiwarter and Saintmont (1908),[2] who state that cats will not breed under restraint.

Anestrous female cats show spontaneous courtship, mating and postcopulatory ovulation, following administration of appropriate gonadotropins (Friedgood, 1939[3]; Windle, 1939[4]); and spontaneous estrus occurs regularly in confined females. Therefore, any obstacle to the successful breeding of cats in the laboratory may be safely referred to some failure on the part of the male.

There is no reason to assume that close confinement affects the male's fertility. Although we have not studied in detail the gonads of males with which we worked, preliminary inspection of the sectioned testes from six males kept in the laboratory for more than a year indicates that spermatogenesis was normal. Ejaculates containing large numbers of motile sperm were obtained from one individual. Furthermore, the single intact female tested with our males became pregnant and delivered a normal litter. These observations, plus the fact that captive males invariably exhibited appreciable increase in body weight and gave other evidence of being in good health, lead us to suspect that the male cat's failure to impregnate the fertile females in the laboratory is due, primarily, to a lack of sexual aggressiveness. This impression is strengthened by the observations of Langley (1911),[5] who reports that "there is great variation in the behavior of males and that, while observers were present, few of those tried would act promptly, or at all. In a considerable number, only one was found which could be relied upon to afford a final test for oestrus."

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[1] Observations here reported constitute part of an experimental investigation supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

[2] **Winiwarter, R., & S. Saintmont.** Nouvelles recherches sur l'ovogenèse et l'organogenèse de l'ovaire des mammifères. Arch. de Biol. **24:** 1–47. 1908.

[3] **Friedgood, H. B.** Induction of estrous behavior in anestrous cats with the follicle-stimulating and luteinizing hormones of the anterior pituitary gland. Am. J. Physiol. **126:** 229–233. 1939.

[4] **Windle, W. F.** Induction of mating and ovulation in the cat with pregnancy urine and serum extracts. Endocrinol. **25:** 356–371. 1939.

[5] **Langley, W. H.** The maturation of the egg and ovulation in the domestic cat. Am. J. Anat. **12:** 139–172. 1911.

In the course of an investigation into the effects of brain injury upon sexual activity, we have studied in some detail the mating performance of seventeen adult male cats. These animals were strays purchased from a source which supplies medical schools in the metropolitan area. Their history is unknown and the size and apparent age varied considerably. After a good deal of experimentation, a training schedule was devised which conditioned the cats so that 15 of the 17 cases mated promptly when confronted with an estrous female. Since all but one of the females used were spayed animals in which estrus was induced by estrogen treatment, pregnancy could not occur. However, as explained above, the males were almost certainly fertile and would have impregnated normal estrous queens.

In most cases, males showed no signs of mating until they had been in the laboratory for at least a month. Two individuals copulated (following training) after approximately two weeks' residence. A preliminary period of three to four weeks, in which the animals are allowed to become accustomed to feeding and being handled, was found to be an important prerequisite to successful sexual conditioning. After the acclimatization period, regular tests were conducted in which the following techniques proved helpful.

1. Only fully receptive females were used as stimulus animals. Tests for receptivity were brief and simple. When the loose skin on the back of the female's neck is grasped firmly in the experimenter's fingers, the receptive female will flex the forelimbs, lowering the anterior portion of the body. When the perineum is tapped gently, the receptive female exhibits treading responses, alternate stepping movements of the hind limbs. When a glass rod is inserted in the vagina the female frequently will growl loudly; and, after the neck grip is released and the glass rod withdrawn, the female displays the characteristic, violent after-reactions which include vigorous rolling and twisting on the floor, repeated licking of the vagina, etc.

2. Males were always taken from their living cages to a special test room, and they were never introduced into this room except upon the occasion of a sex test. Through this procedure, the males apparently came to associate the test room with sexual opportunity.

3. In beginning tests, males were allowed 15 minutes or longer to explore the test room before the stimulus female was introduced. In later tests, after conditioning had become apparent, this preliminary interval was found to be unnecessary.

4. The receptive female was placed in the test room and the observer took his station on the outside of the room where his presence did not distract the animals although their activity was clearly visible to him. In early tests, males responded to the female by thorough investigation, but there was little indication of sexual arousal, and mating was rarely attempted. As soon as it became apparent that the male was not going to copulate spontaneously, the experimenter reentered the test room and proceeded to stimulate the female to exhibit heat behavior.

5. Employing the techniques described above, the experimenter induced the female to crouch, tread, and display after reactions. After this performance had been repeated several times in succession, the male's interest in the female usually increased appreciably, although it was often necessary to continue stimulating the female for half an hour or longer before the male showed any evidence of arousal. Three males mated in their first test after the female had been induced several times to go through her entire mating role. The remaining 12 males did not copulate until this stimulus situation had been presented in several successive tests.

6. Conditioned males were tested regularly at least once each week. It is possible that less frequent testing might have been equally effective; but we found that weekly tests produced highly reliable results. Without exception, after a male mated in two or three preliminary tests, sexual responsiveness continued.

After they had copulated in several tests, the males became extremely reliable in their mating performance. They approached and mounted the female with very little delay; and the frequency of completed matings in a series of one hour tests was highly consistent for each individual.

It seems very likely that the simple procedures outlined above, or some variation of them, are customarily employed by commercial animal breeders; but, inasmuch as no published description of any such methods has come to our attention, it seems worth while to make our experience available to others who may be interested in observing behavior or inducing reproductivity in this species.

FEEDING LABORATORY ANIMALS

By J. K. Loosli

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Proper feeding of laboratory animals is an essential to maintaining a vigorous breeding stock useful in experimental investigation. In the efficient feeding of any species, it is helpful to understand the nutritional requirements for the various body functions, in order that foods may be selected to fully satisfy the varying needs.

More studies have been made with the rat than with any other laboratory animal and its nutritive requirements are more fully understood. Therefore, most space is devoted in this discussion to studies with rats. Limited attention is also given to the rabbit, guinea pig, mouse, hamster, and cotton rat. The nutrients required to prevent specific deficiency diseases are briefly considered and diets suggested which will maintain these animals with essential freedom from deficiencies during growth and reproduction.

NUTRITIONAL REQUIREMENTS

Protein: Proteins are required for the formation of new tissues during growth, for replacement of protein tissues broken down in the normal metabolic processes, and for the synthesis of secretory products, such as milk. The amount of protein needed by an animal clearly varies with the type and extent of the metabolic function involved.

Hogan and Pilcher[1] observed more rapid gains in weight on high protein intakes than on a protein-low diet. Johnson *et al.*[2] also obtained more rapid gains in weight on a diet with 25 per cent protein than on one containing 10 per cent protein. The energy utilization was equally efficient at the two levels of protein intake, but on low-protein the animals stored more fat, while those on high-protein stored more protein and water. Forbes *et al.*[3] present data showing that a diet containing 20 per cent of protein produced more rapid gains in body weight and more efficient energy gain than lower levels, but 25 per cent of protein was not superior to the 20 per cent level. Hamilton[4] found that the growth rate of rats increased as the dietary protein was increased from 4 to 16 per cent, remained constant from 16 to 30 per cent, and decreased at higher levels of protein intake. The appetite of rats fed diets containing less than 16 or more than 22 per cent was adversely affected. He concluded that, for rats under 125 grams in body weight, the minimum protein requirement may be as high as 20 per cent of the dry matter.

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[1] Mo. Res. Bull. **195.** 1933.
[2] Mo. Res. Bull. **246.** 1936.
[3] J. Nutr. **10:** 461. 1935.
[4] J. Nutr. **17:** 565, 582. 1939.

McCoy[5] has presented a preliminary report of a study of the effect of different levels of protein in the diet upon growth, reproduction, lactation and body composition. On *ad libitum* feeding, young rats gained weight more rapidly when fed purified diets containing 25 to 40 per cent of protein than with 15 per cent protein. In paired feeding studies, the rate of growth and the percentage of nitrogen in the animal body paralleled the protein intake. In reproduction studies extending five generations, females on the high-protein diet produced more litters and more young per litter than those on the other diets. As judged by the weight of the young at weaning, the diet containing 25 per cent protein produced the most satisfactory lactation.

For normal growth of rats, the minimum protein appears to be supplied by a diet containing 20 per cent of protein to 125 grams in body weight, and 16 to 18 per cent thereafter. Levels of dietary protein of 30 per cent or higher may be disadvantageous for growth. More data are needed on the requirements for lactation.

AMINO ACIDS: Inasmuch as all tissue protein is made up of at least 22 generally recognized amino acids, it becomes obvious that all of these must be furnished either by synthesis within the body or supplied in the diet of the animal. Thus, the protein requirement actually becomes a need for specific amino acids, as illustrated by the early work of Osborne and Mendel.[6] They clearly demonstrated that the amino acids, lysine and tryptophane, are essential for the growth of rats.

Much of our knowledge regarding the amino acid requirements of rats has resulted from the studies of Rose and associates at Illinois. The results of these experiments demonstrate that 10 amino acids are indispensable for growth of the rat,[7] as shown in TABLE 1.

FAT: In 1929, Burr and Burr[8] first reported a deficiency disease produced by the exclusion of fat from the diet. They showed that, on a fat-low diet, rats develop a scaly condition of the skin, followed by a necrosis of the tail, a degeneration of the kidneys and finally a failure of growth and death. On a fat-low diet, females show irregular ovulation and males may refuse to breed. Hematuria, albuminuria and kid-

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[5] J. Biol. Chem. **133:** XIV. 1940.
[6] J. Biol. Chem. **17:** 325. 1914.
[7] Physiol. Rev. **18:** 109–136. 1938.
[8] J. Biol. Chem. **82:** 345. 1929; **86:** 587. 1930.

TABLE 1
CLASSIFICATION OF THE AMINO ACIDS FOR GROWTH OF RATS

Essential	*Non-essential*
Lysine	Glycine
Tryptophane	Alanine
Histidine	Serine
Phenylalanine	Norleucine
Leucine	Aspartic acid
Isoleucine	Glutamic acid
Threonine	Hydroxyglutamic acid
Methionine	Proline
Valine	Hydroxyproline
Argenine*	Citrulline
	Tyrosine
	Cystine

* Argenine cannot be synthesized at a sufficiently rapid rate to permit normal growth.

ney injury are generally found. Burr and associates[9] found that linoleic and linolenic acids both cured the deficiency symptoms and, more recently, arachidonic acid has been shown to be effective (Turpeinen[10]). Evans and coworkers[11] found successful gestation was not possible on a fat-free diet. In pregnant animals, resorption may occur or the gestation period may be prolonged. Maternal mortality is frequent and any litters born are dead or so weak they soon die. Addition to the diet of large amounts of saturated fatty acids does not improve gestation, whereas unsaturated acids allow normal reproduction. Males develop a sterility which is cured or can be prevented by 50 mg. of unsaturated fatty acids prepared from corn oil.

Martin[12] has tentatively suggested that 30 mg. of methyl linoleate daily per rat will permit optimum growth. Turpeinen[13] obtained maximum growth response in plateaued fat-deficient female rats by feeding about 100 mg. methyl linoleate daily, or 33 mg. of the methyl ester of arachidonic acid. Nunn and Smedley-Maclean[14] observed that arachidonic acid is absent from the liver of rats fed a fat-deficient diet for several months, but it is present following the feeding of methyl linoleate. On a diet lower in fat than previously used, Mackenzie and associates[15] reported that, when 25 mg. of methyl linoleate was fed for as long as 11 months, the development of rats was comparable to those on a stock diet. Reproduction appeared normal, but many of the young died within 3 days after birth. The results of exchanging young from mothers fed low-fat and normal diets suggested that lactation on the low-fat diet is inadequate.

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[9] J. Biol. Chem. **97:** 1. 1932.
[10] J. Nutr. **15:** 351. 1938.
[11] J. Biol. Chem. **106:** 431, 441, 445. 1934.
[12] J. Nutr. **17:** 127. 1939.
[13] J. Nutr. **15:** 351. 1938.
[14] Biochem. J. **32:** 2178. 1938.
[15] Biochem. J. **33:** 935. 1939.

That fat in the diet improves lactation performance has been shown by Maynard and Rasmussen.[16] The beneficial effect of extra fat (corn or cottonseed oil) is evident, even when 125 mg. of ethyl linoleate are fed to the lactating mothers daily (Loosli and associates[17]), but no improvement in lactation performance was evident when fully hydrogenated coconut fat was fed.

Forbes and Swift[18] have shown that a certain amount of fat in the diets of rats is essential for a minimum energy loss as dynamic effect. The addition of fat to a protein-carbohydrate diet decreases the dynamic effect and thus increases the useful energy available to the animal. In the case of chickens (Russell *et al.*[19]), the addition of 4 per cent fat to a low-fat basal diet increased the utilization of carotene and permitted larger liver storage when vitamin A was fed. In rats, there is some evidence that the presence of fat in the diet facilitates utilization of carotene and also of calcium, but other studies have failed to confirm such an effect. The matter cannot be considered as settled.

VITAMIN A AND CAROTENE: Vitamin A is essential for all mammals. It is important for growth, normal vision and the maintenance of normal epithelial tissues. Vitamin A deficiency results in night blindness, keratinization of epithelia, a disturbance of bone growth, sterility and cessation of growth.

Wolback and Howe[20] first clearly demonstrated that lack of vitamin A causes replacement of many different epithelia by stratified keratinized epithelium. Functional failure of glands may result. In the female, sterility may ensue from implantation failure due to keratinization of the uterine epithelium (Evans[21]). Fridericia and Holm,[22] Tansley[23] and others have reported that night blindness is one of the early symptoms of vitamin A deficiency. Mason[24] has shown that vitamin A is necessary for maintenance of normal epithelium in the testis. Mellanby[25] has presented evidence indicating that vitamin A deficiency in young dogs causes overgrowth and deformities of the bones. The overgrowth of bones causes pressure upon nervous tissue indirectly producing degenerative changes which were earlier thought to be a direct effect of vitamin A lack (Irving and Richards[26]). Wolbach and Bessey[27] have shown that in vitamin A deficiency in rats, skeletal growth is retarded earlier than that of the soft tissues in general, including that of the central nervous system, and that the nervous manifestations are due to pressure effects caused by relative overgrowth of the central nervous system. Evidence that a stenosis of the optic canal causes constriction of the optic nerve and blindness in vitamin deficiency of young calves is presented by Moore.[28] Wolbach and Howe[29] and Orten *et al.*[30] show that vitamin A deficiency produces upon the incisor teeth a loss of normal orange pigment, opacity, distortion of shape and exfoliation. Mellanby[31] observed pale and deformed teeth in young rats reared by mothers kept for several months on a diet low in vitamin A. A detailed discussion is given by Schour and Massler.[32]

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[16] J. Nutr. **23:** 385. 1942.
[17] J. Nutr. **28:** 81. 1944.
[18] J. Nutr. **27:** 453. 1944.
[19] J. Nutr. **24:** 199. 1942.
[20] J. Exp. Med. **42:** 753. 1925.
[21] J. Biol. Chem. **77:** 651. 1928.
[22] Am. J. Physiol. **73:** 63, 79. 1925.
[23] J. Physiol. **71:** 442. 1931.
[24] Am. J. Anat. **52:** 153. 1933.
[25] J. Physiol. **94:** 380. 1938.

Vitamin A plays a part in the metabolism of visual purple (Wald[33]). Johnson[34] has shown that severe vitamin A deficiency, in addition to producing night blindness, may result in a structural breakdown of the retina itself in rats.

In 1928, Euler[35] reported that 5 micrograms of carotene daily was enough to permit a resumption of growth in rats suffering from vitamin A deficiency. Moore[36] showed that, as a source of vitamin A, carotene was effective at a level of 4 micrograms daily. Guilbert *et al.*[37] have shown that the minimum requirements of the rat are 18 to 22 units of vitamin A, or 15 to 20 micrograms of carotene per kilogram of body weight. This is about in line with several other species of animals studied, suggesting that vitamin A is concerned with general metabolic activities of the body as a whole. Goss and Guilbert[38] observed that 18 to 22 units (3.8 to 4.6 micrograms) of vitamin A, or 15 to 20 micrograms of carotene per kilo of body weight daily, is necessary to prevent cornification of the vaginal epithelium. No storage of vitamin A in the liver occurred until 80 micrograms of carotene per kilo of body weight were given daily. Cannon[39] has reported that female rats depleted of vitamin A until they showed xerophthalmia or weight loss generally did not mate. Others on the depletion diet, plus 40 micrograms of carotene three times weekly, mated and delivered living young. Lewis *et al.*[40] found 50 I.U. daily were necessary to give a significant amount of vitamin A in the liver of rats. None was found on daily intakes of 10 I.U. or less.

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[26] J. Physiol. **94:** 307. 1938.
[27] Am. J. Path. **17:** 586. 1941.
[28] J. Nutr. **17:** 443. 1939.
[29] Am. J. Path. **9:** 275. 1933.
[30] Proc. Soc. Exp. Biol. Med. **36:** 82. 1937.
[31] Brit. Dental J. **67:** 187. 1939.
[32] The Rat in Laboratory Investigation. Griffith and Farris, Eds., J. B. Lippincott Co., Philadelphia. 1943.
[33] Proc. Nat. Acad. Sci. **25:** 344. 1939.
[34] J. Exp. Zool. **81:** 67. 1939.
[35] Biochem. Z. **203:** 370. 1928.
[36] Biochem. J. **24:** 692. 1930.
[37] J. Nutr. **19:** 91. 1940.
[38] J. Nutr. **18:** 169. 1939.
[39] Proc. Soc. Exp. Biol. Med. **44:** 129. 1940.

VITAMIN D: Vitamin D is related to the utilization of calcium and phosphorus, and it is essential for normal calcification of the growing bone. No amount will compensate for severe deficiencies of calcium or phosphorus, but it helps to alleviate the effect of a wide Ca:P ratio.

Schneider and Steenbock[41] observed that, on a low phosphorus diet (0.57 per cent calcium, 0.04 per cent phosphorus) free from vitamin D, young rats gained weight for 4 to 5 weeks, developed severe rickets and bone deformities and died after 6 weeks. Adding vitamin D caused weight loss when the young were placed upon the diet, and gains were very slow. From tissue analyses and calcium and phosphorus balances, the authors suggest that vitamin D induces utilization of phosphorus for bone growth, thereby depriving the soft tissues of a phosphorus supply, which in turn inhibits growth. Templin and Steenbock[42] have furnished evidence that vitamin D markedly decreases the losses of ash from the bones that otherwise occur in the adult rat on a diet very low in calcium. Nicolaysen[43] has suggested that, in vitamin D deficiency, a decreased calcium absorption results. The increased fecal calcium results in precipitation of phosphorus, rendering it unavailable. Cohn and Greenberg[44] have reported that the absorption of phosphate by rachitic rats is increased only slightly by vitamin D, but the phosphorus uptake in the bone is increased by 25 to 50 per cent. Cox and Emboden[45] have shown with rats that growth, bone calcification and reproduction are excellent when adequate amounts and favorable ratios of calcium and phosphorus are available, despite a very low intake or absence of vitamin D in the diet.

VITAMIN E: Vitamin E is known to be essential for normal reproduction in the rat, mouse, and in poultry. When the factor is absent from the diet of the female, death and resorption of the embryos result. Ovulation and conception is not interfered with. In the male rat, permanent sterility results from degenerative changes of the epithelium of the testes. The early studies have been reviewed by Evans.[46] Mason[24] has carefully differentiated the pathology in vitamin E deficiency from that produced by a lack of vitamin A. Many workers have shown that the growth rate of rats is retarded on a vitamin E deficient diet.[47]

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[40] J. Nutr. **23:** 351. 1942.
[41] J. Biol. Chem. **128:** 159. 1930.
[42] J. Biol. Chem. **100:** 209. 1933.
[43] Biochem. J. **31:** 105, 107, 122. 1937.
[44] J. Biol. Chem. **130:** 625. 1939.
[45] J. Nutr. **11:** 147. 1936.

Rabbits and guinea pigs fed diets containing cod liver oil develop a muscular dystrophy (Madsen *et al.*[48]) which can be cured or prevented by feeding a-tocopheral (Eppstein and Morgalis[49]). A similar dystrophy is shown by rats on vitamin E-low diets, particularly the young suckled by vitamin E-low mothers. Mason and Bryan[50] have shown that the placental transfer of vitamin E is very limited, but that larger amounts pass into the milk.

Evans and Emerson[51] have shown that the amount of vitamin E required by rats for normal reproduction increases with age. Feeding 0.10 mg. of alpha-tocopheral acetate, six times weekly, maintained normality of striated musculature in both males and females. In males, 0.10 mg. would not maintain fertility more than five months, and 0.25 mg. was inadequate after nine months, while 0.75 mg. maintained normal testes and fertility during the sixteen month experiment. Females on 0.10 mg. produced living young for three gestations (11 to 12 months). By eighteen months, orange-brown pigmentation of the uterus occurred in females receiving 0.10 and 0.25 mg. but not in those given 0.75 mg. of alpha-tocopheral acetate six times weekly. Young born of mothers on the 0.10 mg. level exhibited muscle dystrophy and high mortality. On 0.25 mg., the incidence was less until the third gestation, when all young were paralyzed. On 0.75 mg., all young were normal for two litters, but, in the third gestation, a slight temporary dystrophy was seen.

Eppstein and Morgulis[49] have reported that a daily dose of 0.32 mg. of alpha-tocopheral per kilo of body weight will cure muscle distrophy in rabbits.

In the mouse, the administration of 0.5 to 1.0 mg. alpha-tocopheral at the beginning of gestation resulted in normal young in at least 85 per cent of the cases (Goettsch[52]), whereas those without the supplement produced no litters. The second generation males on the vitamin

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[46] J. Am. Med. Assoc. **99:** 469. 1932.
[47] Cited, Ref. [32].
[48] Cornell Univ. Agr. Exp. Sta. Memoir: **178.** 1935.
[49] J. Nutr. **22:** 415. 1941.
[50] J. Nutr. **10:** 501. 1940.
[51] J. Nutr. **26:** 555. 1943.
[52] J. Nutr. **23:** 513. 1942.

E-low diet were fertile and showed no testicular degeneration, as also reported by Bryan and Mason.[53]

THIAMINE: Thiamine occurs both in the free form and as thiamine pyrophosphate or cocarboxylase in foods and in tissues. It functions in the living cell as a coenzyme in carbohydrate metabolism. In a thiamine deficiency, the metabolism of carbohydrate is incomplete and pyruvic acid accumulates in the tissues.

Polyneuritis, cardiac dysfunction, muscular atrophy and anorexia occur in thiamine deficiency. A number of investigators including Davison and Stone,[54] Zimmerman,[55] and Lee and Sure[56] have reported degeneration of the nervous system in vitamin B_1 deficiency, but others have not been able to show nerve changes. Engle and Phillips[57] have shown that in uncomplicated B_1 deficiency there is no degeneration of the peripheral nerves. Lipschits, Potter and Elvehjem[58] reported that thiamine deficiency produces a general disturbance of carbohydrate metabolism rather than lesions of the nervous system. This view is in line with the earlier report of Lohmann and Schuster[59] that cocarboxylase is a pyrophosphoric acid ester of thiamine. Banerji and Harris[60] found that with increasing severity of thiamine deficiency there is greater excretion of bisulfite-binding substances (essentially pyruvic acid). Arnold and Elvehjem[61] demonstrated that the thiamine requirement is directly related to the non-fat calories and that rats grow at a normal rate when the diet contains 80 to 100 micrograms of thiamine per 100 grams of food, or approximately one microgram per gram of non-fat energy supplying food.

Sure[62] found that 10 micrograms of thiamine daily will cure polyneuritis and permit satisfactory growth of rats, whereas normal lactation requires 120 micrograms.

RIBOFLAVIN: In nature, riboflavin may occur, as such, as riboflavin phosphate or as a constituent of specific flavoproteins which function as enzymes in tissue respiration. Enzymes known to contain riboflavin include cytochrome reductase, d-amino acid oxidase and xanthine oxidase.[63] Axelrod and Elvehjem[64] have reported a reduction in the tissue concentration of these enzymes in riboflavin deficient animals.

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[53] Am. J. Physiol. **131:** 263. 1940.
[54] Arch. Path. **23:** 207. 1937.
[55] Schweitzer Archiv. für Neur. und Psychiat. **39:** 195. 1937.
[56] Arch. Path. **24:** 430. 1937.
[57] J. Nutr. **16:** 585. 1938.
[58] J. Biol. Chem. **123:** 267. 1938.
[59] Biochem. Z. **294:** 188. 1937.
[60] Biochem. J. **33:** 1346. 1939.
[61] J. Nutr. **15:** 429. 1938; Am. J. Physiol. **126:** 289. 1939.
[62] Endocrinology **23:** 575. 1938; J. Nutr. **19:** 57. 1940.

Riboflavin deficiency causes retarded growth, dermatitis and cataract in rats and characteristic paralysis in chicks. Day *et al.*[65] observed almost 100 per cent incidence of cataract in rats on a riboflavin deficient diet, which could be prevented or arrested by feeding riboflavin. Bourne and Pyke[66] experienced a much lower incidence of cataract. Baum *et al.*[67] indicate the incidence of cataract in rats is increased if the diet supplies a small amount of riboflavin. Phillips and Engle[68] have shown a neuropathologic condition of the main peripheral nerve trunks in chicks on low riboflavin rations, but the condition was not seen in rats until the deficiency was aggravated by an increased fat content of the diet. On a diet very low in riboflavin (Street *et al.*[69]), dogs exhibit a characteristic collapse, syndrome and sudden death. Prolonged subsistence on a diet low in riboflavin results in neurological abnormalities accompanied by myelin degeneration of peripheral nerves and the posterior columns of the spinal cord. Opacities of the cornea may also occur in the dogs.

Day and Darby[70] have stated that rats receiving 90 to 120 micrograms of riboflavin per week gained weight at a slightly subnormal rate, but reached adult size and remained in apparent health for more than a year. Edgar *et al.*[71] reported that optimum growth is possible on a diet supplying about 40 micrograms of riboflavin per day. Sure[72] concluded about 120 micrograms are needed daily for satisfactory rearing of young.

PANTOTHENIC ACID: Rats placed on diets low in pantothenic acid grow very slowly and develop in a few weeks necrosis of the adrenal cortex (Daft and Sebrell[73]). Loss of hair pigmentation (graying or nutritional achromatrichia) has been observed in many laboratories in piebald rats as shown by Unna *et al.*[74] Graying has also been reported in dogs, guinea pigs and silver foxes (Morgan *et al.*[75]). In deficient animals there is generally a characteristic accumulation and incrustation of a red, fluorescent, porphyrin-containing exudate on the nose, whiskers and fur.

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[63] Hogness, T. R. A symposium on respiratory enzymes. Univ. of Wis. Press, Madison. 1942.

[64] J. Biol. Chem. **140:** 725. 1941.

[65] Am. J. Ophthalmal. **14:** 1005. 1931; J. Nutr. **7:** 97. 1934; **13:** 389. 1937; **15:** 83. 1938.

[66] Biochem. J. **29:** 1865. 1935.

[67] Science **95:** 24. 1942.

[68] J. Nutr. **22:** 345. 1941.

[69] J. Nutr. **22:** 7. 1941.

[70] J. Biol. Chem. **123:** XXVIII. 1938.

[71] Biochem. J. **31:** 879. 1937.

[72] J. Nutr. **19:** 57. 1940.

[73] Pub. Health Report **55:** 1333. 1940.

[74] J. Nutr. **22:** 553. 1941.

[75] J. Nutr. **19:** 233. 1940.

Schaefer *et al.*[76] have produced acute pantothenic acid deficiency in dogs, that is characterized by sudden collapse associated with decreased blood dextrose, increased nonprotein nitrogen and lowered blood chlorides. Severe intussusception in the intestinal tract and fatty livers have also been observed. Phillips and Engle[77] earlier reported specific neuropathologic changes in the spinal cord in chicks suffering from pantothenic acid deficiency. Wintrode *et al.*[78] have found nerve degeneration in pigs fed synthetic diets low in pantothenic acid and other members of the B complex.

The pantothenic acid requirement of dogs is about 0.10 mg. per 100 grams ration (Elvehjem[79]). Sixty micrograms of calcium pantothenate per day prevented graying in rats, but 100 micrograms were necessary for maximum growth (Henderson *et al.*[80]).

Leonards and Free[81] have observed that the rate of intestinal absorption of galactose was 15 per cent more in rats on normal diets than in animals deficient in pantothenic acid. Supplementation of a normal diet with 300 and 1000 micrograms of calcium pantothenate had no effect on the rate of absorption.

The biochemistry of pantothenic acid has been reviewed by Williams.[82]

PYRIDOXINE: Pyridoxine has been shown to be essential in the diet of the rat,[83] the dog,[84] the pig and poultry. A lack of this vitamin causes retarded growth, an acrodynia characterized by edema, swelling and denuding of the paws and areas around the mouth, and frequently thickening of the ears. A microcytic hypochromic anemia is characteristic in dogs. Sullivan and Evans[85] have described the dermal changes as distinguished from those seen in magnesium deficiency. Patton *et al.*[86] have reported the occurrence of convulsive seizures in young rats suckling mothers fed diets deficient in pyridoxine. The literature dealing with dermal changes in deficiencies of pyridoxine and the essential fatty acids is reviewed by McCoy.[32]

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[76] J. Biol. Chem. **143:** 321. 1942.

[77] J. Nutr. **18:** 227. 1939.

[78] Bull. Johns Hopkins Hosp. **67:** 377. 1940.

[79] Hand Book of Nutrition. Am. Med. Assoc., Chicago. 1943.

[80] J. Nutr. **23:** 47. 1942.

[81] J. Nutr. **25:** 403. 1943.

[82] Advances in Enzymol. **3:** 253. 1943.

[83] **Gyorgy** *et al.* Proc. Soc. Exp. Biol. Med. **37:** 313. 1937.

[84] J. Nutr. **16:** 197. 1938; J. Nutr. **21:** 275. 1941; J. Biol. Chem. **142:** 77. 1942.

[85] J. Nutr. **27:** 123. 1944.

[86] J. Biol. Chem. **152:** 181. 1943.

Emerson and Evans[87] have reported uniformly defective sexual behavior in pyridoxine deficient rats. Sure[72] found 10 to 25 micrograms of pyridoxine adequate for growth, but at least 50 micrograms for satisfactory lactation in rats.

BIOTIN: When young rats are fed a diet containing uncooked egg white as the source of protein, there develops a disease characterized by poor growth, generalized pruritic, exfoliative dermatitis, abnormal posture, abnormal gait and hypertonicity. The cutaneous signs described by Boas[88] and later workers are "spectacle eye," progressing to general alopecia, onset of a spasticity and finally death. Lease *et al.*[89] found that the rabbit and the monkey also exhibited a characteristic dermatitis when fed rations rich in egg white. Du Vigneaud *et al.*[90] first identified biotin as the vitamin involved, and Gyorgy *et al.*[91] established the presence of "avidin" as the biotin inactivating factor in egg white. Sullivan *et al.*[92] have suggested that the hypertonicity may be due to irritability caused by severe pruritus, since no anatomical changes in the central nervous system were evident.

Nielson and Elvehjem[93] found that, on an egg white diet, 2 micrograms of biotin per rat daily prevented or cured the "spectacle eye" syndrome. Signs of biotin deficiency do not appear on synthetic diets without egg white.

NIACIN (NICOTINIC ACID): The dog, pig and monkey are the only experimental animals that show typical nicotinic acid deficiency. Niacin is not required, preformed in the diets of rats or chicks (Harris and Raymond[94]), Axelrod *et al.*,[95] Sure.[96] Elvehjem *et al.*[97] showed that blacktongue in dogs could be prevented or cured by niacin. That the silver fox also requires niacin has recently been shown (Hodson and Loosli.)[98]

Nicotinic acid functions as a component of two important coenzymes —coenzyme I, or cozymase, and coenzyme II—which are concerned in both glycolysis and respiration (Elvehjem[99]). In nicotinic acid deficiency, there is a decreased cozymase content of the liver and muscle tissue.

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[87] Am. J. Physiol. **120:** 352. 1940.
[88] Biochem. J. **18:** 422. 1924.
[89] Biochem. J. **31:** 433. 1937.
[90] Science **92:** 62. 1940.
[91] Science **93:** 477. 1941.
[92] Bull. Johns Hopkins Hosp. **70:** 177. 1942.
[93] Proc. Soc. Exp. Biol. Med. **48:** 349. 1941.
[94] Biochem. J. **33:** 2037. 1939.
[95] J. Biol. Chem. **131:** 85. 1939.
[96] J. Nutr. **19:** 57. 1940.
[97] J. Biol. Chem. **123:** 137. 1938.
[98] Vet. Medicine **37:** 470. 1942.
[99] Physiol. Rev. **20:** 249. 1940.

VITAMIN K: A deficiency of vitamin K is manifested by a marked tendency to severe hemorrhage associated with an increase in the blood-clotting time. The symptoms are prevented or cured by vitamin K. Several investigators have shown the synthesis of vitamin K by the intestinal flora of rats (Dam *et al.*[100]; Greaves and others[101]), thus explaining the usual failure to produce the deficiency in this species. Greaves found that only 12 of 77 animals reared and maintained on a vitamin-K-free diet exhibited subnormal prothrombin values and hemorrhagic tendencies. Bile-fistula or jaundiced animals showed marked bleeding tendencies associated with low prothrombin, which was cured by feeding vitamin K. Day *et al.*[102] have presented data showing the cecum of the rat is an important site of vitamin K synthesis, but that this vitamin can also be formed in other parts of the intestinal tract. Cecectomized rats showed very low incidence of hypoprothrombinemia, whereas operated animals fed one per cent sulfasuxidine, exhibited a very high incidence. On the sulfasuxidine diet, the addition of p-aminobenzoic acid markedly reduced the incidence of hypoprothrombinemia, thus counteracting the effect of sulfasuxidine on vitamin K synthesis in the intestinal tract. This is in agreement with earlier studies (Elvehjem[103]).

CHOLINE: For many years choline has been known as a component part of the phospholipid lecithin, but its functional importance in nutrition was not apparent until Best and Huntsman[104] demonstrated its role in the prevention of fatty livers.

Choline is now generally considered an important member of the vitamin B complex, although Jacobi *et al.*[105] have demonstrated that it may be synthesized in the rat. The function of choline is related to the mobilization of fatty acids in the body. Du Vigneaud *et al.*[106] observed that the methyl groups of choline as well as those of methionine and betaine are transferable in the animal body. McHenry[107] states that choline may function in at least three ways: to stimulate the formation of phospholipids, to make possible the production of acetyl choline, or to supply labile methyl groups.

Griffith[108] reported fatty degeneration of the liver, hemorrhagic renal lesions, ocular hemorrhages and regression of the thymus within ten days after the rats had been placed on a choline-low but otherwise efficient diet. He found 1.0 mg. of choline chloride daily was needed to prevent renal lesions in young rats and 2 to 3 mg. to prevent fatty livers. Sure[109] reported 15 mg. choline chloride usually gave satisfactory lactation.

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[100] Biochem. J. **31:** 22. 1937.
[101] Am. J. Physiol. **125:** 423, 429. 1939.
[102] J. Nutr. **26:** 585. 1943.
[103] Chem. and Eng. News **21:** 853. 1943.
[104] J. Physiol. **75:** 405. 1932.
[105] J. Biol. Chem. **138:** 571. 1941.
[106] Biological Symposia **5:** 234. 1941.
[107] Biological Symposia **5:** 177. 1941.
[108] J. Nutr. **22:** 239, 1941; **19:** 437. 1940; J. Biol. Chem. **131:** 567. 1939.

Ascorbic Acid: Deficiencies of ascorbic acid can be demonstrated only in man, monkey and the guinea pig. Other species have the ability to synthesize this vitamin. The pathology of vitamin C deficiency has been reviewed in detail by Dalldorf.[110] Five to ten milligrams of ascorbic acid appear to be sufficient to protect the guinea pig against symptoms of scurvy.

Other Vitamins: Several groups of workers have recently shown that the guinea pig requires two or more dietary factors in addition to all the known vitamins discussed in this paper and also inositol, folic acid and p-amino benzoic acid. Hogan and Hamilton[111] obtained fair growth and survival of guinea pigs fed purified diets supplemented with the known vitamins and factors from dried yeast and liver. Sober *et al.*[112] found that essential unknown vitamins are supplied by yeast, dried grass and whole milk. Wooley[113] and Kuiken *et al.*[114] show that guinea pigs need three factors in addition to the known vitamins. The rat apparently does not require a dietary source of these factors.

Calcium and Phosphorus: Calcium and phosphorus are discussed together because of the interrelationships of these mineral elements in nutrition. This relationship is well illustrated by the report of Bethke, Kick and Wilder[115] who suggest the Ca:P ratio is more important in determining the adequacy of the intakes than are the actual amounts of the elements. As they increased the Ca:P ratio from 1:1 to 5:1, a progressive decrease occurred in growth, bone ash and the inorganic phosphorus in the blood. Decreasing the Ca:P ratio from 1:1 to 0.25:1 decreases the growth rate and the serum calcium, but has only a slight effect upon the bone ash. They observed best growth when dietary calcium was 0.69 per cent and phosphorus 0.56 per cent. Cox and Imboden,[116] however, found that successful reproduction and lactation in the rat were dependent upon both the actual level and the Ca:P ratio in the diet. The best results were obtained on a diet containing 0.59 per cent of each calcium and phosphorus (a daily intake of 42 mg.). Reproduction was poor when the diet contained 2.45 per cent of either element. Hubbell *et al.*[117] have suggested a salt mixture which allows rapid growth with an average daily intake of 50 mg. calcium and 35 mg. phosphorus. Sherman and Booher[118] and Sherman and Campbell[119] reported that, with diets containing 0.42 per cent phosphorus, increasing the calcium progressively from 0.16 to 0.50 per cent permitted more rapid calcification of bones, earlier maturity, and deferred senescence. Van Duyne *et al.*[120] found diets containing 0.64 to 0.80 per cent calcium optimum in promoting nutritional well-being as shown by full-life experiments in three generations.

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[109] J. Nutr. **19:** 71. 1940.
[110] The Vitamins. Am. Med. Assoc., Chicago. 1939.
[111] J. Nutr. **23:** 533. 1942.
[112] J. Nutr. **24:** 503. 1942.
[113] J. Biol. Chem. **143:** 679. 1942.
[114] J. Nutr. **27:** 385. 1944.
[115] J. Biol. Chem. **98:** 389. 1932.
[116] J. Nutr. **11:** 147. 1936.

Day and McCollum[121] reported that young rats on a diet containing 0.4 per cent calcium and 0.017 per cent phosphorus, but otherwise adequate, grow slowly for 5 to 6 weeks, then decline in weight and soon die. Extreme rarefraction of the bones occurs, accompanied by progressive disability in walking, standing and breathing. A loss of calcium occurs in the urine. A smaller loss of phosphorus (largely in the urine) suggests that part of this element mobilized from the bones is used by the soft tissues for growth. This severe phosphorus deficiency does not have any significant effect upon the metabolism of sodium, potassium, or magnesium. Boelter and Greenberg[122] found rats reared from weaning on a low-calcium diet (10 mg. per 100 gm. food) failed to mate, and mothers on calcium-low diet did not lactate normally.

Most satisfactory growth, reproduction and livability are obtained when the diet of rats contains about 0.6 per cent calcium and 0.5 per cent phosphorus, although good results can be obtained at appreciably lower levels.

MAGNESIUM: Sullivan and Evans[123] have recently produced uncomplicated magnesium deficiency in rats. Two or three days after weaning rats were placed on the magnesium-deficient diet, they were nervous and hyperirritable. After the first week, spontaneous or induced convulsions occurred. Some animals succumbed immediately after the convulsions, others survived several seizures. The first cutaneous sign was erythema. The paws soon became swollen and red. Growth was normal for the first two or three weeks. As the deficiency progressed, the animals became weak, less irritable and listless. After the sixth to eighth week, there was nutritive failure and variable superimposed illness. Occasionally, there was loss of hair and dermatitis. The dermal changes are distinguishable from pyridoxine deficiency. There is no relation between magnesium deficiency and that of the vitamin B complex.

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[117] J. Nutr. **14:** 273. 1937.
[118] J. Biol. Chem. **93:** 93. 1931.
[119] J. Nutr. **10:** 363. 1935.
[120] J. Nutr. **21:** 221. 1941.
[121] J. Biol. Chem. **130:** 269. 1939; J. Nutr. **20:** 181. 1940.
[122] J. Nutr. **26:** 105. 1943.
[123] J. Nutr. **27:** 123. 1944.

These gross symptoms are generally similar to those earlier described by Kruse, Orent and McCollum[124] in rats and dogs; Tufts and Greenberg[125] and Watchorn and McCance[126] in rats.

The serum magnesium falls in magnesium deficient animals, as does the percentage of magnesium in the skeleton and soft tissues. The calcium content of the soft tissues and bones increases. Calcification of the kidneys and blood vessels have been reported. Snyder and Tweedy[127] have found that there is a marked fall in serum phosphatase activity associated with the decline in magnesium. There is a disturbance in the calcification of the teeth (Becks and Furuta[128] and Irving[129]).

Tufts and Greenberg[130] found that 5 mg. of magnesium per 100 grams of diet (4 mg. per kilo body weight) is about the minimum requirement for growth. Reproduction was normal at this level of intake, but suckling young showed signs of deficiency.

MANGANESE: In 1931, it was shown that manganese was essential in the diet of the rat (Orent and McCollum[131]) and the mouse (Kemmerer *et al.*[132]). Since that time, manganese has been reported to be essential for normal reproduction, lactation, bone formation, growth and the activity of certain enzymes. Workers are agreed that manganese deficiency produces testicular atrophy, sterility and retarded growth. Kemmerer *et al.*,[132] Waddell *et al.*,[133] Skinner *et al.*[134] and Boyer *et al.*[135] report disturbances of the estrous cycle resulting in sterility, whereas Orent and McCollum,[136] Daniels and Everson[137] and Shils and McCollum[138] observed no such effect. Manganese is important for normal bone development in chicks (Wilgus *et al.*,[139] and Caskey[140]). The studies of Barnes *et al.*[141] and Shils and McCollum[142] suggest the same is true for rats. Boyer *et al.*[135] reported a decreased arginase activity in manganese deficiency. Shils and McCollum[142] found manganese-deficient young may show loss of both equilibrium and coordination. High dietary calcium accentuates the severity of manganese deficiency in both chicks and rats. High intakes of manganese retards the growth rate of rats.[142] Adding 0.005 to 0.05 per cent of manganese to a low-manganese diet maintained fertility in males.[136] A daily intake of 0.5 to 0.8 mg. appears to be adequate for reproduction.

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[124] J. Biol. Chem. **96:** 519. 1932; Am. J. Physiol. **101:** 454. 1932.
[125] J. Biol. Chem. **122:** 693. 1937–38.
[126] Biochem. J. **31:** 1379. 1937.
[127] J. Biol. Chem. **146:** 639. 1942.
[128] J. Am. Dent. Assoc. **26:** 883. 1939.
[129] J. Physiol. **99:** 8. 1940.
[130] J. Biol. Chem. **122:** 715. 1937–38.
[131] J. Biol. Chem. **92:** 651. 1931.
[132] J. Biol. Chem. **92:** 623. 1931.
[133] J. Nutr. **4:** 53. 1931.
[134] Am. J. Physiol. **101:** 591. 1932.
[135] J. Biol. Chem. **143:** 417. 1942.
[136] J. Biol. Chem. **98:** 101. 1932.
[137] J. Nutr. **9:** 191. 1935.
[138] J. Nutr. **26:** 1. 1943.
[139] J. Nutr. **14:** 155. 1937.
[140] Proc. Soc. Exp. Biol. Med. **44:** 332. 1940.

Smith *et al.*[143] have shown that manganese deficiency in rabbits results in a decreased breaking strength, weight, density, length and ash content of the humeri. Deformed front legs is the most evident gross symptom. The manganese requirement for growth of rabbits appears to be about 1.0 mg. daily.

IRON AND COPPER: The history of the development of our knowledge regarding the role of iron in hemoglobin formation has been reviewed by Robscheit-Robbins.[144] Hart *et al.*[145] first reported evidence that copper was also essential for hemoglobin formation.

Josephs[146] reported that copper does not effect iron retention, but is concerned in hemoglobin formation. Elvehjem and Sherman[147] and Cunningham[148] found that, when iron is fed to the anemic rat in the absence of copper, the iron content of the liver and spleen increases but there is no rise in the hemoglobin level. Stein and Lewis[149] concluded that copper has two roles in blood formation: a catalytic action on hemoglobin formation and a stimulating effect on erythropoiesis. Cohen and Elvehjem[150] presented evidence suggesting that copper was concerned in cytochrome formation. They also showed that feeding copper or copper and iron markedly increased the oxidase content of the livers of anemic rats, but iron alone had no effect. Smith and Medlicott[151] have recently reported that deficiency of iron or copper, or both, produces a microcytic hypochromic anemia. Feeding pure iron to milk-anemic rats leads to a significant increase in the mean cell volume. Feeding copper to milk-anemic rats produced a rise in the erythrocyte count which was not accompanied by an increase in hemoglobin. This evidence corroborates the view of Stein and Lewis[149] that copper has a stimulating effect on erythropoiesis or on the release of erythrocytes from the bone marrow.

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[141] Proc. Soc. Exp. Biol. Med. **46:** 562. 1941.
[142] J. Nutr. **23:** 445. 1942.
[143] Arch. Biochem. **4:** 281. 1944; and unpublished data.
[144] Physiol. Rev. **9:** 666. 1929.
[145] J. Biol. Chem. **77:** 797. 1928.
[146] J. Biol. Chem. **96:** 559. 1932.
[147] J. Biol. Chem. **98:** 309. 1932.
[148] Biochem. J. **25:** 1267. 1931.
[149] J. Nutr. **6:** 465. 1933.
[150] J. Biol. Chem. **107:** 97. 1934.
[151] Am. J. Physiol. **141:** 354. 1944.

Mitchell and Miller[152, 154] found 0.25 mg. of iron daily maintained normal hemoglobin when sufficient copper was present and that with less than 0.1 mg. of copper hemoglobin synthesis was retarded.

Hart *et al.*[145] have shown that iron and copper are the only constituents of liver needed for maximum hemoglobin formation by milk anemic rats.

There is evidence[152, 153, 154, 155, 156] that 0.25 mg. of iron and about 0.05 mg. of copper per day is adequate to maintain hemoglobin values or to regenerate hemoglobin rapidly in young anemic rats. A daily intake of 5 mg. of iron and 0.5 mg. of copper appears adequate for normal reproduction and lactation (Daniels and Everson[157]) but these values are probably more than the minimum requirements.

Potassium: A detailed picture of uncomplicated potassium deficiency in rats has been presented by the studies of Orent-Keiles and McCollum.[160] When young rats were fed a diet containing only 0.01 per cent of potassium, growth continued, but at a slow rate, and the length of life did not seem to be affected. There was a roughness and thinning of the fur, and the animals showed an extreme alertness and marked pica. Ovulation was irregular and at a slower rate with occasional cessation of estrus. Sexual maturity was delayed. The potassium in the muscles, heart, and kidneys was lowered, while sodium increased. All of the potassium-deficient animals exhibited necrosis of the cardiac musculature and damage to the renal tubular epithelium, but other tissues were normal according to a report by Follis *et al.*[161] Cardiac and renal hypertrophy was observed. These studies confirm earlier experiments with rats by Schrader *et al.*[162] and by Thomas *et al.*[163] that cardiac and renal injuries result from potassium deficiency. According to Heppel,[164] serum potassium falls to about one half normal in potassium-deficient rats, and the chloride is about 15 per cent lower.

Heppel and Schmidt[165] found that pregnancy was possible when the diets of rats contained 0.010 to 0.015 per cent of potassium, but the

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[152] J. Biol. Chem. **92:** 421. 1931.
[153] J. Biol. Chem. **104:** 217. 1934.
[154] J. Biol. Chem. **85:** 355. 1929–30.
[155] J. Nutr. **4:** 469. 1931.
[156] J. Nutr. **5:** 295. 1932; **23:** 47. 1942.
[157] J. Nutr. **9:** 191. 1935.
[160] J. Biol. Chem. **140:** 337. 1941.
[161] Am. J. Path. **18:** 29. 1942.
[162] J. Nutr. **14:** 85. 1937.
[163] Yale J. Biol. Med. **12:** 345. 1940.
[164] Am. J. Physiol. **127:** 385. 1939.
[165] Univ. of Calif. Pub. Physiol. **8:** 189. 1938.

mothers lost body tissue and the young were eaten at birth. During the lactation period, a dietary content of 0.58 per cent of potassium usually resulted in some storage. Miller[166] earlier stated the minimum potassium requirement for normal growth of rats was about 15 mg. for males and 8 mg. for females (0.55 to 1.44 gm. potassium per kilo of ration). The maintenance requirement is not more than 2 mg. daily.

Sodium and Chlorine: Orent-Keiles *et al.*[167] found that retardation of growth, disturbances in reproductive functions, lesions of the eyes, and death resulted when rats were restricted to a diet which contained only 0.002 per cent of sodium. A detailed, histological study of Follis *et al.*[168] failed to reveal any specific tissue changes other than those in the ocular apparatus.

On a sodium-deficient diet, rats were in negative sodium balance, but retention of potassium and magnesium increased so that the acid-base balance was not appreciably disturbed (Orent-Keiles and McCollum[169]). Kahlenberg *et al.*[170] have shown that sodium deprivation did not affect the ability of rats to digest and absorb protein and energy. There was, however, a depressed appetite, increased heat production, and decreased body storage of protein and energy. A similar depression of the utilization of protein and energy has been shown to occur when rats are fed a diet deficient in chloride (Voris and Thacker[171]). Differing from sodium deficiency, however, the drastic restriction of chloride intake does not appear to cause any pathological tissue changes nor death. Marquis,[172] and Greenberg and Cuthbertson[173] reported that rats continued to grow at a reduced rate on a chloride-low diet. The latter authors showed that the urinary excretion of chloride fell rapidly and that rats receiving only about 1 mg. of chloride daily were still in positive chlorine balance.

Cobalt: It has not been definitely established that cobalt is required by rats or other laboratory animals, although such a need may be indicated on the basis of studies with sheep and cattle. If rats have a need for cobalt, the amount required is less than 0.4 microgram daily (Underwood [174]). Orten *et al.*,[175] Stare and Elvehjem[176] and Meyer

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[166] J. Biol. Chem. **55:** 61. 1923; **70:** 587. 1926.
[167] Am. J. Physiol. **119:** 651. 1937.
[168] Arch. Path. **33:** 504. 1942.
[169] J. Biol. Chem. **133:** 75. 1940.
[170] J. Nutr. **13:** 97. 1937.
[171] J. Nutr. **23:** 365. 1942.
[172] Compt. rend. soc. biol. **128:** 449. 1938.
[173] J. Biol. Chem. **145:** 179. 1942.
[174] Nutr. Abs. and Rev. **9:** 515. 1940.
[175] J. Biol. Chem. **96:** 11. 1932; **99:** 457. 1932–3; Am. J. Physiol. **114:** 414. 1935–6.
[176] J. Biol. Chem. **99:** 473. 1932–3.

et al.[177] have shown that feeding cobalt to rats (0.5 mg. daily) produces a marked polycythemia. It has been suggested that the cobalt produces an increase in the rate of formation of hemoglobin and erythrocytes.

ZINC: Studies at Wisconsin have shown that zinc is necessary for the growth of rats. On a nearly zinc-free diet, growth is retarded, and the development of the fur is interfered with (Stirn *et al.*[178]). The amount of zinc in the body, and especially in the bone, teeth and blood is reduced. Hove *et al.*[179] found that 40 micrograms of zinc per day prevented the deficiency.

IODINE: Iodine is essential in the diet in order to prevent enlargement of the thyroid gland (goiter). Remington and associates[180] have studied the iodine requirement of rats. The thyroid greatly increases in total fresh and dry weight in iodine deficiency, and the iodine content markedly decreases. They found that 1 to 2 micrograms of iodine per rat daily will prevent thyroid enlargement.

Chesney *et al.*[181] reported goiter in rabbits fed a diet consisting solely of cabbage. These goiters were associated with a lowering of the metabolic rate, and feeding iodine raised the metabolic rate and prevented thyroid hyperplasia. Baumann *et al.*[182] confirmed the findings and concluded that cabbage contains a goiterogenic substance which acts by depleting the thyroxin store of the thyroid gland.

DIETS FOR LABORATORY ANIMALS

The most successful results in raising laboratory animals are obtained in practice when a variety of good quality natural food materials are available. Because of the wide distribution of most nutrients in common foods, there are innumerable combinations of foodstuffs which would adequately supply the nutritional needs of laboratory animals. A few such combinations of natural food materials will be suggested.

It has been possible to devise diets of highly purified ingredients which permit excellent growth in rats and mice. While instances of reproduction have also been recorded, animals have not been maintained for many generations upon purified diets as a final test of their completeness. Furthermore, upon such diets, only limited success has been achieved with rabbits and guinea pigs.

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[177] J. Biol. Chem. **94:** 117. 1931–2.
[178] J. Biol. Chem. **109:** 347. 1935.
[179] Am. J. Physiol. **119:** 768. 1937; **124:** 750. 1938.
[180] J. Nutr. **6:** 325. 1933; **15:** 539. 1938.
[181] Bull. Johns Hopkins Hosp. **43:** 261. 1928.
[182] Proc. Soc. Exp. Biol. Med. **28:** 1017. 1931.

In 1930, Maynard[183] reported a ready-mixed, commercially available stock diet for rats which gave satisfactory results during growth and reproduction. The mixture was composed of linseed oil meal, 300 pounds; ground malted barley, 200; wheat red dog flour, 440; dried skim milk, 300; oat flour, 300; yellow corn meal, 400; steamed bone meal, 20; ground limestone, 20; and salt, 20 pounds. Extra vitamins A and D were supplied twice a week but no green feed or other supplements were fed. This same mixture has served as a stock diet in the Cornell rat colony until the present time (October, 1944) and it is considered to be an adequate diet. It has also been successfully used during many generations for the mouse and hamster, and for the rabbit, when fed along with hay; and for the guinea pig, when extra roughage and vitamin C was supplied. Recent tests in our laboratory and a comparison of the growth and reproduction of other rat colonies (Smith[184]) clearly demonstrated that the calf meal, as the only food, is far from optimum for rats.

Mendel and Hubbell[185] fed their rats, in addition to the calf meal described above, other supplements which have improved growth and reproduction. Nursing mothers and young rats under 6 weeks of age received a "paste food" consisting of casein 25 per cent, whole milk powder 25, wheat embryo 20, and lard 30 per cent. The calf meal and the paste food were both supplied *ad libitum*. Each rat also received 1 gm. of dried yeast daily except Sunday, and those without paste food were given 3 gm. wheat embryo per week. No "green" food of any sort was used. The comparative rates of growth of rats fed calf meal only and those fed extra supplements are shown in FIGURE 1. Data on reproductive efficiency are presented in TABLE 2.

The marked improvement in the rate of growth, the larger adult size and the superior reproduction are most probably the result of dietary improvement due to the paste food and yeast.

Sherman and associates[186] have shown that a diet made up of about five parts whole wheat and one part whole milk powder with 2 per cent of salt is adequate for growth and reproduction of rats during many generations. Nevertheless, such a diet is not optimum, for they have observed that supplements which furnish extra calcium, riboflavin or protein resulted in more rapid growth, earlier maturity, longer reproductive life, larger adult size and longer life. Adding extra fat resulted in a slightly slower growth rate but also an increased length of life.

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[183] Science **71:** 192. 1930.
[184] U. S. D. A. and Cornell University. Unpublished data.
[185] J. Nutr. **10:** 557. 1935.
[186] J. Nutr. **14:** 609. 1937; **16:** 603. 1938.

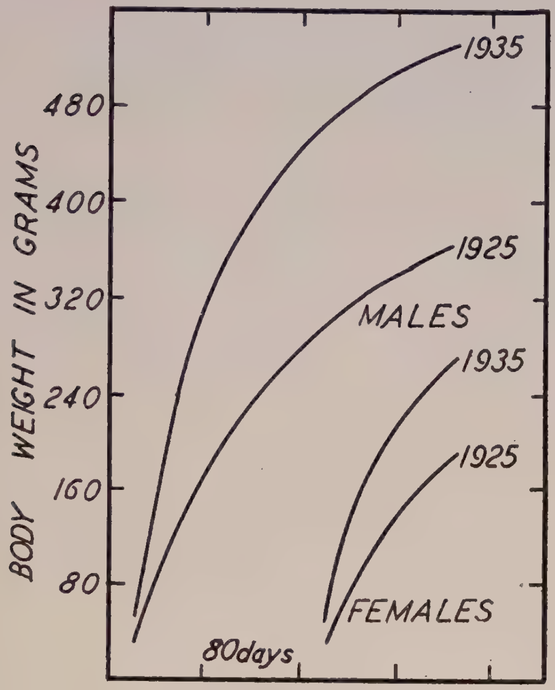

FIGURE 1.
Growth of rats on calf meal (1925) and on calf meal plus the "paste food" supplement.

TABLE 2
REPRODUCTIVE PERFORMANCE UNDER DIFFERENT DIETARY CONDITIONS

	Fertile matings	No. in litter	Birth wt.	Weaned	Weaning Wt.		Av. daily gain to 100 days	
					Males	Females	Males	Females
	%		gm.	%	gm.	gm.	gm.	gm.
1919 to 1925	68	6.4	—	76	31	30	2.1	1.6
1925 to 1935	93	9.6	5.8	90	48	47	4.0	2.5

That appreciable fat in the diet of rats is essential for optimum lactation performance is clearly demonstrated by the studies of Maynard *et al.*[187] Adding 10 to 20 per cent of fat to the diet of females resulted in a marked increase in the growth-rate of the young during the suckling period. When fat was added to calf meal or to a low-fat synthetic diet to increase the amount to 10 per cent, young rats gained, as an average, 1.5 grams during the first 17 days, as compared with about 1.0 gram without the extra fat. At weaning (21 days), young on high-fat weigh 40 to 45 grams as compared with 25 to 30 grams for young fed the low-fat diets.

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[187] J. Nutr. **23:** 385. 1942; **28:** 81. 1944.

The importance of this rapid gain in weight for rats to be used in assay studies has been stressed by Anderson and Smith.[188] These authors have reviewed the reports since 1906 showing the average weights of the albino rat. During this time, a marked increase in the rate of growth has occurred. They express the view that improved feeding practice has been a major cause of the more rapid growth rate. Selected data which illustrate the increased growth rate of albino rats are shown in TABLE 3.

TABLE 3
THE AVERAGE GAIN IN WEIGHT OF ALBINO MALE RATS

Date and Reference	Body weight at 100 days of age	Average daily gain
	gm.	*gm.*
1906 (Donaldson *et al.* [189])	165	1.65
1915 (King [190])	200	2.0
1928 (Smith and Bing [191])	315	3.2
1935 (Mendel and Hubbell [185])	—	4.0

In a recent survey Smith[184] has compared the rate of growth and the reproductive efficiency of a number of rat colonies. The litter size and the body weights of males from these colonies are shown in TABLE 4.

The striking difference in the performance of these rat colonies is extremely interesting. It is not possible to indicate with certainty the relative importance of genetic differences and of feeding and management practices as causes of these variations. When it is recalled, however, that the Connecticut colony (Mendel and Hubbell[185]) increased in breeding efficiency and rate of growth from the level of the other colonies shown in table 4 to its present high rating (see table 2), at least, largely as the result of a dietary change, it appears worth while to consider the diets used in these colonies. A comparison of the diets is shown in TABLE 5.

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[188] Am. J. Physiol. **100:** 511. 1932.
[189] Boas Anniversary Volume **5.** 1909. New York.
[190] Anat. Record **9:** 751. 1915.
[191] J. Nutr. **1:** 179. 1928.

Table 4

Litter Size and Body Weight of Different Rat Colonies

Colony	Young in 1st litter	Weight of males		
		At weaning	At 100 days	Maximum wt.
	No.	*gm.*	*gm.*	*gm.*
Columbia	6.8	52.6[2]	274	388
Connecticut	9.5	51.8[1]	358	531
Cornell	6.2	29.7[1]	234	361
Penn State				
Pied	7.4	—	—	—
Albino	7.9	—	—	—
Wild	7.4	—	—	—
U. S. D. A.	6.4	65.6[2]	276	434

[1] 21 days of age.
[2] 28 days of age.

Table 5

A Comparison of Diets Fed to Different Rat Colonies

	Columbia	Connecticut[1]	Cornell	Penn State[2]	U.S.D.A.[3]
Wheat	5	—	—	18	33
Yellow corn	—	20	20	67	33
Malted barley	—	10	10	—	—
Wheat red dog	—	22	22	—	—
Oats or oat flour	—	15	15	2	10
Skim milk powder	—	13	13	—	10
Whole milk powder	1	—	—	2	—
Soluble blood flour	—	2	2	—	—
Wheat bran	—	—	—	1	—
Meat scrap	—	—	—	2	—
Alfalfa leaf meal	—	—	—	2	3
Casein	—	—	—	—	4.5
Lard	—	—	—	—	5.5
Salt	1.6	1	1	0.4	0.5
$CaCO_3$	—	—	—	0.6	0.5
Ferric citrate	—	—	—	0.04	—

[1] Nursing dams and young to 6 weeks of age are also given a "paste food" consisting of casein, 25; whole milk powder, 25; wheat embryo, 20; lard, 30. Each rat is given 1 gm. of yeast daily and 3 gm. wheat embryo, each week, when they receive no "paste food."

[2] Each rat receives about 10 cc. whole milk daily and, except nursing dams, 5 cc. tomato juice 3 times weekly.

[3] The rats are fed whole milk 3 times weekly.

Preliminary studies now in progress at Cornell suggest that much of the difference between the five rat colonies studied by Smith[184] probably can be attributed to dietary differences.

Excellent growth can be achieved when rats are fed diets made up of highly purified food ingredients and supplemented with crystalline vitamins. Reproduction upon such a dietary regime has been less successful. Vinson and Cerecedo[192] have recently reviewed earlier studies and reported purified diets which maintained growth, reproduction and lactation through four generations. Selected data on reproduction and lactation presented by these authors for Wistar strain of rats are shown in TABLE 6.

TABLE 6
REPRODUCTION ON HIGHLY PURIFIED DIETS

Generation	Litter size	Average weaning wt.
	no.	*gm.*
Parent	6.7	38.3
F$_1$	5.3	33.8
F$_2$	4.0	34.2
F$_3$	4.0	29.6
Controls on Purina dog chow	7.0	34.5

While growth was rapid upon the diets employed, lactation was poor. The authors suggest that some substance (lactagogue), causing increased milk production, is lacking in the purified diets. Such a subsubstance seems to be present in yeast. The data also show that reproduction is less efficient than upon the control diet.

Morse and Schmidt[193] have shown that nitrogen is lost during gestation on a synthetic diet, but stored on a diet of natural feeds. Nitrogen losses during lactation were greatly increased on the synthetic diet.

THE COTTON RAT will probably find more use as a laboratory animal and, therefore, the nutritive requirements of this animal are of interest. Jungeblut[194] has shown that this species does not require a dietary source of ascorbic acid. McIntire, Schweigert and Elvehjem[195] list the B vitamin requirements in micrograms per 100 grams of diet as follows: thiamine 150, pyridoxine 100, pantothenic acid 800, riboflavin between 80 and 300, nicotinic acid less than 2,500, inositol less than 100,000, choline less than 100,000, and other factors present in liver extract.

THE HAMSTER: Coopermann, Waisman and Elvehjem[196] recently studied the nutritional requirements of the golden hamster. They fed a basal diet of sucrose 72, casein 18, salts 5, corn oil 2, cod liver oil 2, and wheat germ oil 1, with 1 drop of haliver oil every two weeks. Satisfactory growth and instances of reproduction were observed when thiamine, riboflavin, calcium pantothenate, pyridoxine, sodium para-aminobenzoate, inositol, choline, and biotin were supplied. Their data support the view that nicotinic acid is not essential. Routh and Houchin[197] had earlier reported that the hamster needed nicotinic acid. Vitamin C is not a dietary essential. On a biotin-deficient diet Cooperman *et al.*, observed a characteristic dermatitis at the corners of the mouth.

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[192] Arch Biochem. **3:** 389. 1944.
[193] Proc. Soc. Exp. Biol. Med. **56:** 57. 1944.
[194] J. Nutr. **20:** 427. 1940.
[195] J. Nutr. **27:** 1, 1944.
[196] Proc. Soc. Expt. Biol. Med. **52:** 250. 1943.

A diet of natural food materials which is satisfactory for the albino rat will probably also meet the needs of the cotton rat and the hamster.

Rabbits and guinea pigs: Hogan and Ritchie,[198] in attempts to rear rabbits and guinea pigs on purified diets, found they failed to grow and survive on diets which were satisfactory for rats. Madsen *et al.*,[48] and Davis *et al.*[199] obtained fair growth on certain purified rations. Shen[200] observed approximately normal growth of guinea pigs and survival up to two years on a diet of casein 15, starch 35.5, sucrose 15, cellulose 15, agar 5, salts 4.5, cottonseed oil 5, and yeast 6, supplemented with irradiated yeast, carotene and tomato juice. A number of the females bred, but they generally aborted or delivered dead young.

Kohler *et al.*[201] reported a "grass juice factor" which is essential for growth of guinea pigs. Cannon and Emerson[202] obtained data which they interpreted as a confirmation of the existence of this dietary essential.

Sober *et al.*,[203] using guinea pigs, obtained most rapid growth and longest survival on a purified diet containing sucrose 62, casein 30, salts 4, corn oil 4, thiamine 250 mg., pyridoxine 250, riboflavin 350, pantothenic acid 1000, niacin 5 mg. and choline 400 mg. The diet was supplemented with yeast 16 per cent, dried grass 16 per cent, and 20 ml. whole milk. Each animal was given 10 mg. ascorbic acid daily and 1.0 mg. a-tocopherol acetate and 4 drops haliver oil weekly. On this diet the animals survived at least 7 weeks and gained an average of 3.8 gm. per day. Hogan and Hamilton[204] found guinea pigs would not grow or survive long on purified diets containing only the known crystalline vitamins. Dried yeast and a water extract of liver contain the unidentified essentials. These authors doubt the existence of an essential "grass juice factor" for the guinea pig. Reproduction was obtained in rabbits when extracts from yeast and liver were fed. Wooley[205] and Kuiken *et al.*[206] reported evidence suggesting that guinea pigs require three more dietary factors than do rats and mice. Two of these factors are furnished by linseed oil meal.

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[197] Federation Proc. **1:** 191. 1942.
[198] Missouri Agr. Exp. Sta. Bull. **219.** 1934; **370:** 23. 1936.
[199] Cornell Agr. Exp. Sta. Memoir **217.** 1938.
[200] Thesis, Cornell University, Ithaca, N. Y. 1939.
[201] J. Nutr. **15:** 445. 1938.
[202] J. Nutr. **18:** 155. 1939.
[203] J. Nutr. **24:** 503. 1942.
[204] J. Nutr. **23:** 533. 1942.

Mannering *et al.*[207] confirm the presence in linseed oil meal of two factors which are essential for guinea pigs. They show data supporting the view that essential factors are also furnished by solubilized liver powder and grass juice powder.

It has not been conclusively established that rabbits and guinea pigs require all known members of the vitamin B complex, but it appears certain that they need two or three vitamins which are not now available in crystalline form. Guinea pigs need vitamin C to prevent scurvy while rabbits appear to be able to synthesize ascorbic acid. In selecting stock rations, it is imperative that some green feed be constantly available for guinea pigs to supply ascorbic acid.

Bischoff and Sansum[208] maintained rabbits for two years on alfalfa. When barley was the only food, rabbits developed fatty livers and nephritis. Hogan and Ritchie[198] fed their stock rabbits whole oats and alfalfa hay, plus about 75 cc. of whole milk of lactating females.

At Cornell, rabbits and guinea pigs have been successfully maintained on the calf meal (TABLE 5) fed to rats and hamsters. Good quality fine stemmed legume or mixed hay is also fed *ad libitum* and guinea pigs receive green feed (grass, carrots, cabbage, lettuce or sprouted grains) two or three times weekly. Good results have also been obtained with a number of dry dog foods which are generally available. A very simple mixture of whole wheat 2, whole oats 2, and linseed oil meal 1 (pelleted or pea size coke), as suggested by Templeton *et al.*,[209] has given fully as satisfactory results as the more complicated feeds. With the latter mixture, it is advisable to supply a mineralized salt block in addition to the hay. Slanetz[210] has recently studied the nutritive quality of several commercial feeds especially prepared for laboratory animals.

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[205] J. Biol. Chem. **143:** 679. 1942.
[206] J. Nutr. **27:** 395. 1944.
[207] J. Biol. Chem. **151:** 101. 1943.
[208] J. Nutr. **5:** 403. 1932.
[209] U. S. D. I. Conservation Bull. **25.** 1942.
[210] Am. J. Vet. Res. **4:** 182. 1943.

DISCUSSION OF THE PAPER

Dr. George R. Cowgill (*Professor of Nutrition, Yale University*):

Dr. Loosli's paper surveys very well the individual dietary factors important for successful feeding of various species of animals. A few comments can be made regarding some of these factors.

Proteins and Amino Acids—Some interesting observations have been made of animals subsisting on diets containing less than the optimal amount of protein. Students of hormone problems have a useful tool in the young female rat forced to grow while subsisting on a diet containing about 4 to 6 per cent protein, instead of the 12 to 18 or more per cent used by most investigators. In our laboratory at Yale some years ago, Drs. Orten and Smith (1937)[1] observed that young female rats fed diets containing 3.5 per cent lactalbumin grew somewhat, but definitely failed to mature sexually. Many interesting observations were made on these animals. The gonads were able to respond to an injection of pregnant mare's serum, proving that the condition was one of lack of gonadotropic stimulation rather than actual injury by the dietary regime. We may speculate on the possibility that this represented a failure of the pituitary gland to grow properly in the face of a limited supply of certain essential amino acids. It is known that the hormones elaborated by the pituitary gland are protein in nature and that these proteins contain at least some of the amino acids known to be essential for nutrition of the rat. We may suppose that, in the face of a limited supply of certain essential amino acids, the growing pituitary gland is forced to compete with other structures of the body with the result that it fails to make the customary growth, and, as a result of this, many other structures which depend on the pituitary gland fail to grow properly. This would explain the sexual immaturity observed in these animals. We see in observations of this sort an illustration of how the diet can be an important factor for students of the physiology of sex to consider in their researches.

Calories—Dr. Loosli did not mention the fact that, broadly speaking, animals eat calories, although this is implied in much of his discussion. It is this phenomenon of "eating calories" that makes it very necessary for us, when performing feeding experiments and endeavoring to make quantitative observations, to see that the food intake is measured, if possible. Since an animal eats enough food to meet the energy requirement, it will eat considerably more of a low-fat diet in order to secure those needed calories than it will of a high-fat diet. This indicates the importance in feeding experiments of knowing not only the percentage composition of the diet but the energy value as well. Incidentally, I might add that it is as a rule more difficult to secure accurate intake data when feeding a low-fat diet because of its powdery nature, than it is when feeding a high-fat diet which is likely to be paste-like and more solid and therefore more difficult for the experimental animals to scatter. When feeding a powdery diet, various ingenious devices have been employed to keep the animals from scattering the food.

Fat—Dr. Loosli ably summarized the literature on the importance of fat in the diet. A word or two might be said regarding some practical aspects of this matter. Most dry animal feeds that are not marketed in a sealed container—a can for example—are very low in fat. This is due to the tendency of high-fat mixtures to turn rancid and therefore to spoil before they can be used by the purchaser.

In our own feeding experiments with dogs, we have become impressed with the importance of knowing, for each animal, what we call the Nutritive Index. By the Nutritive Index, we mean the ratio of the cube root of the body weight to the length of the animal, *i.e.*, $\frac{\sqrt[3]{\mathrm{WgLT}}}{\mathrm{LA}}$ (Cowgill and Drabkin, 1927[2]; Cowgill, 1928[3]). We have measured this Nutritive Index in many dogs and satisfied ourselves that the maximum value is about 0.34, when weight is expressed in grams and length in centimeters and the weight is the maximum possible for the unit length, for example, the value for an extremely obese dog. This maximum value, namely 0.34, may be compared with the value yielded by the animal in question. If this animal is not as completely filled out in all three dimensions, so to speak, for its length, then the value will be less than 0.34. A group of dogs was allowed to have all that it wished of diets of known composition. After a period, each animal put itself into such a nutritive state that the value of this ratio in every case was very close to 0.30. We, therefore, take 0.30 as the value representing a good state of nutrition that the dog will endeavor to reach if allowed all it wishes of good food. If a dog is fasted sufficiently, the value obviously drops considerably below 0.30.

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[1] **Orten, Aline U., & A. H. Smith.** The effect of dietary protein on endocrine function and on the blood picture of female rats. Am. J. Physiol. **119:** 381. 1937.

[2] **Cowgill, G. R., & D. L. Drabkin.** Determination of a formula for the surface area of the dog together with a consideration of formulae available for other species. 1927.

[3] **Cowgill, G. R.** The energy factor in relation to food intake: experiments on the dog. Am. J. Physiol. **85:** 45. 1928.

Some years ago, we had occasion to use our Nutritive Index in an interesting way on dogs being used by Dr. Hansen for studies of fat metabolism. Interesting values for blood lipids had been secured. Dr. Hansen had tried to make his animals more or less equal at the beginning of the study by fasting each of them for a short period. However, some of the animals were in excellent nutritive condition when such a fast began, whereas others were not so well nourished. Obviously, a short fast does not seriously affect an animal that has a large fat reserve; a dog with little fat reserve, when subjected to a fast, quickly begins to burn his own body tissue. Many of Dr. Hansen's data, apparently, could not be explained. When, however, he determined the Nutritive Index of each animal, it was perfectly obvious what had happened and it was clear that the dogs were not the same just because of the short fast.

We have consistently used this principle of Nutritive Index in our work with dogs. Whenever dogs are put on metabolism experiments, where they are expected to eat all of the food offered and to be similar with respect to what we like to regard as a "known nutritive state," the Nutritive Index is determined and then the animal fasted or liberally fed until the Nutritive Index approximates a value slightly below 0.30. Then the particular experiment of interest is started. By this procedure, we have been able to eliminate a large amount of variation in metabolic data yielded by our dogs. Perhaps, this has some application to other species. So far as I know, this point has not been tested.

VITAMIN A AND CAROTENE—Dr. Loosli has pointed out the importance of vitamin A in nutrition and its relation to the provitamin carotene. One might call attention to the fact that different species show different degrees of utilization of carotene in studies where vitamin A is the variable of interest. This fact should be taken into account.

As Dr. Loosli pointed out, vitamin A deficiency is characterized by changes in the epithelial tissues. This has a bearing on the problem of infections in the colony. Some years ago, with my colleague, Dr. H. M. Zimmerman, studies were made on vitamin A deficient rats to determine whether certain lesions develop in the nervous system. Many groups of animals were started, but they failed to survive long enough for such lesions to develop. We, therefore, isolated the animals in a private room and instructed the assistant who took care of the animals to use what amounted to an aseptic technique as far as possible. As a result, we had perfect success. The animals did not die of infections, but developed a very definite and characteristic paralysis. Treatment with large doses of carotene resulted in restoration of growth, a good hair coat, a return to a general nutritive condition, but no cure of the paralysis. This experience taught us the importance, in any vitamin A deficiency studies, of protecting the animals against ordinary infections. Presumably, the fall in resistance to infection is related to the keratinizing of the epithelial cells lining the upper respiratory passages.

VITAMIN E—This vitamin has acquired a significance in nutrition somewhat different from that originally attributed to it. We now know the chemical structure of vitamin E and some of its important chemical properties. We know that it is a very valuable anti-oxidant. According to the most recent work of Hickman and associates (1944),[4] it can serve to protect many other factors, notably vitamin A, carotene, and even ascorbic acid from oxidation. Other workers have shown that batches of experimental diets made up in any quantity for laboratory work, if allowed to become rancid, will lose their content of vitamin E. In experimental work, therefore, it is important to take measures to avoid the development of such rancidity. There is a very practical and important question here to which the investigator must give careful attention, if he is to be sure that any vitamin E added to the food mixture will still be there when the animal actually eats the food.

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[4] **Hickman, K. C., M. W. Kaley, & P. L. Harris.** Covitamin studies. I. The sparing action of natural tocopherol concentrates on vitamin A. J. Biol. Chem. **152:** 303. 1944; II. The sparing action of natural tocopherol concentrates on carotene. J. Biol. Chem. **152:** 313. 1944; III. The sparing equivalence of the tocopherols and mode of action. J. Biol. Chem. **152:** 321. 1944.

B-Complex Factors—The latest interesting findings bearing on these factors, that might be commented upon here, relate to the production of several of the B vitamins in the intestine by bacteria. This helps to explain the observations made many years ago that animals allowed access to their feces can get along with smaller amounts of B-complex vitamins in their food. We now know that the feces contain many of these factors and also that the amounts present in the feces can be influenced by the composition of the diet. It is for this reason, among others, that students of the B-complex problems almost invariably house their animals in raised-screen bottom cages, through which the feces can escape from the animal. Many years ago, we had occasion to measure the amount of vitamin B needed by young rats housed in the old style cage, as compared with that required when the animals were in cages provided with screen bottoms. The requirement for B proved to be roughly twice that when the old-style cage was used. The bearing of this on many feeding experiments seems obvious. One might also cite much literature showing that the carbohydrate in the diet can affect the amounts of various members of the B vitamins present in the feces. Apparently, the carbohydrate which has the least effect and is, therefore, an important one to use in experimental studies of this general problem is sucrose. When starch, or dextrin, or lactose is used, analyses of the feces show interesting differences in amounts of various B-complex factors present.

Mention might also be made of the fact that the ingestion of raw egg-white results in a binding of the biotin present in the food as well as that produced by the intestinal bacteria, with the result that the feeding of a diet containing an appreciable amount of raw egg-white can result in the signs of biotin deficiency. Also, many sulfa drugs have been found to inhibit the growth of certain intestinal bacteria. By the use of such drugs, it has even been proven possible to produce in the animal, under certain conditions, the characteristic signs of deficiencies of certain of the B-complex factors.

Vitamin C—Dr. Loosli has pointed out that not all species require this factor. Man, the anthropoids and the guinea pig are species that are unable to synthesize ascorbic acid sufficiently to meet their requirements. Time will not permit a discussion of many interesting phases of vitamin C functions in nutrition. It does seem pertinent, however, to comment here on the use of guinea pigs in the laboratory. I have been surprised at the number of "practical" animal raisers who believe the old claim that a guinea pig does not need to be provided with water, as such, "because this animal eats such large amounts of water-rich leaves—lettuce, etc.—enough water can be obtained from such materials." One guinea pig breeder said that, when he put water in the cage, the results were very bad because the animals would get wet. It has been our experience that, when the cage is provided with a proper drinking fountain, so that the animal can get its water without getting a bath, so to speak, they drink really large amounts. This indicates that they certainly have a water requirement that should not be neglected and which can be easily taken care of by suitable cage arrangement. This merely confirms what any student of nutrition can quickly prove by simple calculations and considerations of metabolism. I doubt whether any competent research worker with guinea pigs neglects to provide water in this fashion. Apparently, this false notion prevails almost entirely among the so-called "practical" animal raisers.

Mineral Nutrients—In considering this group of dietary factors it seemed appropriate to mention the work of Richter (1942–43)[5] at Johns Hopkins University. Dr. Richter, as you know, has been interested in what experimental animals choose to eat when allowed to make many choices of one kind or another. A study of such responses leads to the conclusion that any marked and unusual

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[5] **Richter, C. P.** Total self regulatory functions in animals and human beings. The Harvey Lectures Series **38:** 63–103. 1942–1943.

"appetite" means a special requirement for that factor which the animal is endeavoring to meet. People who operate animal colonies and who desire to study this kind of a problem will find much of profit in the cage devices and experimental arrangements devised by Dr. Richter.

General Comments—It might be pertinent here to make some reference to all-glass cages which many laboratories have used to advantage, particularly when studying problems involving trace elements of one kind or another. Our laboratory has been interested in problems relating to lead. We have used an all-glass cage for young rats and, by this device, together with the feeding of diets extremely low in lead, have been able to get animals with a lead content lower than any reported in the literature. We have even used special stainless steel cages for similar experiments on puppies and with equally satisfactory results.

Not all workers are aware of the importance of checking the effect of some simple laboratory manipulation of a food or the diet on its chemical composition. One illustration of what I have in mind will suffice. Studies have been made of the mineral composition of cereal grain and other plants grown under special conditions. It has been found that, if the wheat or other material that has been carefully grown under carefully controlled conditions is ground up in an ordinary mill made of iron, just to facilitate the chemical analysis, very appreciable amounts of iron can be added to the material being analysed. Erroneous conclusions that have been published regarding the iron content of such materials have been readily explained in this way. One wonders whether this sort of error may not have crept into other food analyses reported in the literature. When careful analyses are being made of substances present in very small amounts, one should not ignore the possible effects of various simple laboratory procedures or special manipulations of the material.

Dr. M. H. Ross (*Biochemical Foundation, Newark, Del.*):

We have had to feed large numbers of dogs and lately have been having trouble getting meat. Perhaps some one has some suggestions to offer.

Dr. Ellis J. Robinson (*American Cyanamid Co., Stamford, Conn.*):

We have been maintaining 40 to 50 dogs and have had a good supply of meat up to the present.

Dr. C. A. Slanetz (*College of Physicians and Surgeons, Columbia University, New York, N. Y.*):

During the last 8 years we have had 200 dogs and have fed them common dog meal. I would suggest you get in touch with the American Medical Association.

Dr. B. J. McGroarty (*Sharpe and Dohme, Glenolden, Pa.*):

We have been very well supplied with meat for our dogs used in work on rabies.

Dr. Ross:

Manufacturers change the formula for the constituents of the food according to what the market has available. However, the protein and carbohydrate content of the formula may remain the same. Many of the products are imported from Canada.

Dr. Robinson:

We have been following the hematology of rats fed the same common diet and for over five years the whole pathological picture has remained very much the same. One needs to know the additional weight of the animal when starting an experiment and how the amount of food will change the requirements.

Dr. Cowgill:

I am very much surprised that people think meat is essential in the diet of dogs. At Yale, dogs have been fed, for many years, on highly purified diets without any meat and good results have been obtained.

Dr. Clarence A. Mills (*College of Medicine, University of Cincinnati, Cincinnati, O.*):

I should like to confirm Doctor Cowgill's remark on maintaining dogs on a simple diet. We have maintained dogs on a synthetic diet and they take it as well as meat and often prefer it. This is not true of cats. Cats are harder to keep than other animals. One must find a diet that is palatable to the cat as cats will often go on a hunger strike. I would like to hear more discussion on the subject.

Dr. T. F. Zucker (*College of Physicians and Surgeons, Columbia University, New York, N. Y.*):

There is not much information on work with cats but it has been found that the animals' diet can be improved by adding miscellaneous items such as B complex (wheat germ). Carotene is an ineffective element in the diet of the cat. Early work on the diet of the cat has shown that diet deficiencies are directly related to the growth and stature of the cat.

INFECTIOUS DISEASES OF LABORATORY ANIMALS

By Herbert L. Ratcliffe

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Whatever the field of investigation, or the immediate problem to be studied, one may say that the experimental biologist more often than not has hope rather than assurance that his animal subjects are healthy. When one is concerned only with short-term studies, perhaps the source and care of experimental animals need not be considered so completely. Yet, certainly, any experiment demands that variables be reduced as much as is possible, which, in turn, demands standardized animals maintained under standardized conditions. This goal may not be completely attainable, animals being what they are. One obstacle is the considerable number of infectious agents to which laboratory animals are susceptible.

Under natural conditions, the species from which laboratory animals are derived are subject to many of the same infections that occur in captivity, yet they survive and multiply. One wonders, at times, whether or not efforts have been misdirected. Possibly, colonies of laboratory animals should be fully exposed to infections, until, by selection, highly resistant strains are obtained. Certainly, present-day efforts to exclude infections develop highly susceptible races.

Nevertheless, the chief concern of this review will be the more important infectious disease of the following species of animals: rats, mice, guinea pigs, rabbits, dogs, cats, monkeys, and canaries. To a large extent, housing and care of colonies of these animals are modified by the necessity of guarding against infections. Hence, these phases of the topic will be considered only as they apply to control of contagious disease.

Location of animal-rooms and their construction, the size and arrangement of cages, the temperature at which quarters are kept, and hygienic considerations have varied widely as different laboratories have sought to meet the needs of one or another species. Certainly, considerable variation is possible without neglecting essentials.

This much is certain, animal-rooms should be proof against wild mice and rats, and against insects. They should be adequately lighted, ventilated and heated. Walls and floors should withstand thorough washing, with satisfactory drains to allow rapid drying. Tables, cageracks and other installations should be so constructed that they do not interfere with cleaning.

When colonies are to occupy more than one room, each room should be so constructed that it may be completely isolated. Then, outbreaks of infectious disease may be checked, sick animals isolated and new arrivals quarantined. When animals are used in experiments involving infectious agents, they should, of course, be completely isolated from other susceptible stock and all possible sources of contact controlled.

Construction and arrangement of cages, obviously, must be governed by the species of animal and purpose for which it is intended. Whatever the type and size of the cages, they should be large enough to permit freedom of movement, allow ready access to animals without undue danger of escape, be easily cleaned, and withstand repeated sterilization. Laboratory supply houses offer many types of cages, few of which meet these requirements.

Rodents, rabbits, and monkeys should be provided with some form of bedding. Straw, saw-dust, shavings, and grain-wastes are commonly employed. Whatever the material used, it must be regarded as a vehicle by which insects, arachnids, and other pests may be introduced into the colony. Mite infection of the skin of mink has been traced to contaminated rice hulls.[1] Saw-dust, wood-shavings, or cut-paper may contain "bed-bugs" (*Cimex*), or dog and cat feces. "Bed-bugs" suck blood and may cause considerable debility. Ova of tapeworms contained in cat or dog feces readily infect rabbits, rats, and mice. Therefore, bedding should be sterilized and stored in safe containers. How frequently bedding material need be changed will, of course, depend upon the species of animal, and its space allowance.

As in all animals, recognition of disease in laboratory animals requires some experience. One cannot stress too strongly the fact that evidences of illness are most quickly recognized by those who know the appearance and behavior of normal animals. Considerable time may be well spent simply in observing normal animals, for colony caretakers may sometimes overlook abnormalities which, if recognized early, often allow more effective control of epidemic disease.

With few exceptions, the onset of acute infectious disease is marked by inactivity, loss of appetite, rough coat, and dull, partly closed eyes. These signs merely warn of possible danger, but are no guide to its specific nature. So, too, others changes that may be noted in the animal, either before or after death, rarely identify the disease.

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[1] **Bushnell, F. R., & O. J. Humon.** Fur Trade J. **21:** 5–6. Canada. 1944.

Usually, specific diagnosis requires identification of the pathogenic organism by microscopic examination of appropriate material, by bacteriologic techniques, or by animal inoculation.[2]

Descriptions of diseases of laboratory animals are widely scattered and often confused. Fortunately for me, this is not the first effort to assemble and digest this information. I am especially indebted to K. F. Meyer,[3] whose review of the infectious disease of rabbits, guinea pigs, rats and mice has simplified my task immeasurably, and to J. H. Dingle,[4] whose more recent discussion of the infectious disease of mice should be carefully studied by those interested in this species. Both of these papers contain vivid descriptions of disease processes and clear directions for diagnosis and control, and both have extensive bibliographies. The present discussion duplicates much that is contained in these papers, and omits consideration of many of the less common diseases that are covered by these authors.

The various species of laboratory animals that are considered in this review are susceptible to a host of infections. Some of these are specific to the species, others have a much wider range. The following descriptions of disease are arranged in the order of their apparent significance.

BACTERIAL DISEASE

(Ref. 5, 6, 7, 8, 9)

1. **Salmonellosis.** Bacteria of the *Salmonella* group are potentially, if not actually, the most important causes of disease among laboratory animals, especially the small rodents. In most instances, outbreaks are caused by two species, *S. typhimurium* or *S. enteritidis,* singly, together, or in combination with other organisms of this group. Both species are as widely distributed as their natural hosts, wild mice and rats, and are as ever-present. Guinea pigs, rats, mice, canaries, and other birds are highly susceptible. Rabbits may be infected by contact with mice or rats that are carriers, and, on occasion, the organisms

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[2] **Topley, W. W. C., & G. S. Wilson.** The Principles of Bacteriology and Immunity. 2nd. ed. William Wood & Co. Baltimore. 1937.

[3] **Meyer, K. F.** The Newer Knowledge of Bacteriology and Immunology: 607–638. Univ. of Chicago Press. 1928.

[4] **Dingle, J. H.** Biology of the Laboratory Mouse: 380–474. Blakiston Co., Philadelphia. 1941.

[5] **Lovell, R.** Vet. Rec. **12:** 1052–1065. 1932.

[6] **Hagan, W. A.** The Infectious Disease of Domestic Animals. Comstock Publishing Co., Ithaca, N. Y. 1943.

[7] **Jaffe, R.** Anatomie und Pathologie der Spontanerkrankungen der kleinen Laboratoriumstiere. Springer, Berlin. 1931.

[8] **Beaudette, F. R.** J. Am. Vet. Med. Assoc. **68:** 642–643. 1926.

[9] **Ratcliffe, H. L.** The Rat in Laboratory Investigations: 456–471. Lippincott Co., Philadelphia. 1942.

have been isolated from cases of acute intestinal disease of dogs and monkeys.[5]

Both wild mice and rats, as well as laboratory stocks of these animals, are common carriers of *S. typhimurium* and *S. enteritidis,* but female guinea pigs may fill the role also; in which case, they serve mainly as a continued source of infection for their colony. Epidemics of salmonellosis in guinea pigs are commonly associated with the breeding season.

The usual route of *Salmonella* infection is by mouth. Contaminated food is the common vehicle. Organisms may enter the colony in food to which wild mice and rats have had access, or in new stock that is added without adequate quarantine, and again the colony may maintain its own sources of infection in mice, rats, or guinea pigs which carry inapparent disease.

When introduced into a susceptible stock, *Salmonella* infections follow the pattern of any acute infectious disease. Some animals die within 48 to 72 hours after exposure, others after several days or weeks. Some recover after more or less prolonged disease, and a few have unapparent infections. Mortality rates vary, but often are higher than 75 per cent of the colony.

Salmonella infections are not distinctive, either *ante mortem* or at autopsy, but must be differentiated from other diseases by bacteriological examinations of the feces before death, or of the liver and spleen after death. After an incubation period of three or six days, signs of acute disease make their appearance. Mice and rats usually develop conjunctivitis and diarrhea, and diarrhea may occur in other species also; but, more commonly, this disease is subacute or chronic, with signs of illness in keeping with the course of the disease. In guinea pig colonies, the disease may make its appearance only during the breeding season, many animals aborting and dying of purulent endometritis.

In animals dying of the more acute types of salmonellosis, one finds only congestion of the viscera, slight enlargement of the liver, spleen and mesenteric lymph nodes, and early catarrhal inflammation of the intestine. When the disease has progressed more slowly, loss of weight may be pronounced. In any event, the most significant pathological changes are limited to the abdominal viscera. The liver, spleen, and mesenteric lymph nodes are greatly enlarged and contain many small pale grey areas of necrosis. Or one may find larger, yellowish abscess-like areas in the spleen and lymph nodes of the mesentery. Usually, the intestinal wall is thickened and injected, and its lymphatic tissue is hyperplastic, and contains yellowish foci of necrosis. Ulcerations of the mucosa of the lower ileum and caecum may be found also. These lesions are fairly uniform in all species that are susceptible to *Salmonella* infection, but the degree to which they are developed depends largely on the duration of the disease. In addition to these changes in the abdominal viscera, it is said that guinea pigs also develop endometrial infection and pneumonia, lesions that can follow infection by many other organisms.

Prevention and control of *Salmonella* infections is difficult. The organisms are so wide spread and may be introduced so easily, that only a carefully developed routine will maintain colonies of mice, rats, guinea pigs, and canaries free of infection. Once infection is established in a colony, destroying the herd and obtaining clean stock is the only method by which its eradication may be guaranteed. If the value of the strain of animals justifies the effort, the entire colony may be divided into small, isolated groups. Then, those in which the disease continues may be destroyed, and other groups tested for fecal carriers.[4] Fecal carriers occur most commonly among rats and mice, but fecal examination of guinea pigs and rabbits is not reliable. Skin testing has been used instead.[3]

Whether or not it may be possible to eradicate *Salmonella* infections in stocks of valuable animals, by use of one of the new antibiotic substances, remains to be determined.*

2. **Pasteurellosis.** Two forms of pasteurellosis occur in laboratory animals, one caused by infection with *Pasteurella aviseptica,* and the other by *P. pseudotuberculosis.* A striking feature of *P. aviseptica* infection in some species is its rapidly fatal course. In such animals, wide-spread capillary hemorrhages and congestion of viscera are conspicuous features. Hence, its designation, "hemorrhagic septicemia." *P. pseudotuberculosis* infection, usually, is a more chronic disease accompanied by the formation of circumscribed tubercle-like nodules in lymph nodes, lungs, and spleen. Because of this lesion, the disease has been called "pseudotuberculosis," a rather unfortunate term, since other organisms cause the development of similar abscesses.

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*In reference to control of *Salmonella* infectious disease, Dr. P. A. Mattis has called my attention to the use of sulfasuxidine and sulfthalidine in intestinal infections. Available publications do not mention tests against paratyphoid organisms, but these drugs may be fed to rats and mice at 2–4% levels in the diet, without evidence of injury [9a, 9b].

[9a] **Thorp, T. S., V. J. Pisciotta, & Cora B. Grundy.** J. Am. Vet. Med. Assoc. **104:** 274–278. 1944.

[9b] **Mattis, P. A., W. M. Benson, & E. S. Koelle.** J. Pharm. and Exp. Thera. **81:** 116–132. 1944.

A. Hemorrhagic septicemia. *P. aviseptica,* the cause of "hemorrhagic septicemia" is a parasite of many species of wild and domesticated animals. Some texts list almost as many specific names for this organism as it has hosts. Thus, its nomenclature has been confused, but there seems to be only a single species. Among laboratory animals, rabbits, mice, and canaries are highly susceptible. Guinea pigs and rats are more resistant, but endemic and epidemic infections have occurred in both species, chronic infection being limited to the nasal passage.

Among laboratory animals, *P. aviseptica* infections probably are best known in rabbits in which a subacute or chronic respiratory disease, known as "snuffles," is the common manifestation. Less frequently, colonies of guinea pigs and rats, also, may carry chronic infections, the organisms being limited to the nasal passages. In mice and canaries, the disease occurs only as epidemics.

P. aviseptica is spread mainly by contact with carriers which discharge the organism in nasal and ocular secretions, but the organisms can be airborne or be carried in food. Crowding and unhygienic conditions obviously promote infections, and rapid spread seems to enhance the virulence of the organism.

In the more susceptible species, *P. aviseptica* infections may cause death before any but the earlier signs of sickness are noted. At *post mortem* examination, all viscera are congested and numerous capillary hemorrhages are seen on serous surfaces.

Less rapidly fatal infections are more common in rabbits, guinea pigs and rats. The acute forms of "snuffles" in the rabbit is characterized by purulent rhinitis and conjuctivitis. The nose and eyes are encrusted with exudate and breathing is labored. The animals are weak, appetite is lost, and emaciation is rapid. At autopsy, one finds purulent bronchitis, broncho-pneumonia, hemorrhagic pleural exudate and sometimes pericarditis. In rabbits which pass into the chronic form of the disease, the rhinitis continues. The animals are emaciated, lack appetite, and cough and sneeze. Autopsy of cases of this form of pasteurellosis reveals more or less extensive pneumonia and abscesses in the subcutaneous tissues, or, if chronic, "snuffles" may be a prolonged process with little or no pulmonary involvement, the infection being confined to the nasal passages.

Guinea pigs react much as do rabbits to *P. aviseptica,* some dying of the acute septicemic disease, others showing respiratory infection. In severe respiratory infections, one finds pneumonia, and hemorrhagic and fibrinous pleuritis, and pericarditis; but, in this species, the infection also spreads to the peritoneal cavity and uterus, and causes enlargement of the spleen.

P. aviseptica is a small gram-negative rod. It grows readily on ordinary media at a wide range of temperature. Diagnosis of infections is not difficult, especially during epidemics, when highly virulent strains of the organism are most often encountered. Subcutaneous injection of a small amount of the heart's blood of a suspected animal into mice usually causes death in less than 24 hours. Blood smears from the inoculated animal contain the organism in great numbers. When colored by Wright's or Giemsa's stain or by good quality methylene blue, the organisms appear darker at each end than in the central part. That is, they show bipolar staining. Isolation of the organism from chronic cases is not so certain, either by culture or animal inoculation, because carrier strains may be relatively avirulent.

Control of *P. aviseptica* infections is best accomplished by selecting stocks of rabbits and guinea pigs that are immune to "snuffles." Any additions to the colonies naturally should be quarantined and carefully examined for signs of the disease. Meyer[3] has outlined a routine that has given satisfactory control.

B. PSEUDOTUBERCULOSIS. *P. pseudotuberculosis* is a parasite of guinea pigs and sometimes of rats, in which it may cause epidemic disease. Infections of other animals are sporadic and usually inapparent.

The natural route of infection with *P. pseudotuberculosis* is the intestinal tract. Affected guinea pigs lose weight, develop diarrhea and die in three or four weeks. Autopsy reveals greatly enlarged lymph nodes in the peritoneal cavity, and in the retro-peritoneal and inguinal regions.

These nodes contain large and small abscesses filled by thick pus. Lymphatic tissue of the gut wall likewise contains many abscesses, and the liver and spleen are enlarged and studded with pale grey nodules which elevate the capsule. Sometimes also the abscesses are found in the lungs. Sporadic cases in other species usually are found by accident when the animal is examined after death. In such cases, the lesion usually consists of single nodules in the lungs or abscessed lymph nodes in the peritoneal cavity.

In the typical lesion, this organism is commonly found within cells. It may be identified tentatively in smears from the abscess by its characteristic bipolar staining, and it grows readily on simple media at a wide range of temperature.

So little is known of the epidemiology of this infection that recommendations for its control cannot be given. Obviously, its discovery, however, in a colony of guinea pigs would warrant their sacrifice and replacement by clean stock.

3. **Pseudotuberculosis of Mice.** *Corynebacterium kutscheri* (*murinum*). This disease of mice is infrequent and chronic, characterized by tubercle-like lesions in the lungs accompanied by pleural effusion. Similar tubercle-like lesions may be found in the liver, lymph nodes of the cervical region, the mediastinum, the mesentery, and isolated nodules in the spleen and kidneys. The organism can be recovered from any of the lesions. The chief interest in this disease is its similarity to Salmonella infections.

4. **Infectious Catarrh.** Chronic respiratory disease of rats is relatively common in many colonies. The disease involves the nasal passages, the middle ear, and the lungs. A high percentage of rats over 600 days of age show some degree of pneumonia. A similar disease occurs in mice also, and, in some instances, has been rapidly progressive.[10] The first sign of this condition in rats may be disturbances of equilibrium. More often, animals first appear unthrifty, after which breathing becomes increasingly labored. In one epidemic among mice, the first signs of illness was described as "chattering,"[10] followed by the development of rhinitis and pneumonia.

Many species of bacteria have been isolated from the nose, ears, and lungs of rats and mice affected by this disease, but none of them appears to be the primary cause. The recent work of Nelson,[10, 11] has shown that these organisms are contaminants and that the disease is caused by minute gram-negative coccobacilliform bodies which can be cultivated only in tissue culture. These reproduce the disease upon intranasal inoculation.

This infection is spread by contact early in the life of the animal. At least two methods to control it have been devised. One is by selective breeding for resistant strains.[12] The other is by using healthy foster-mothers for new-born young of diseased parents.[13]

5. **Tuberculosis.** Sporadic cases of tuberculosis may occur in colonies of guinea pigs and rabbits, but, under usual circumstances, these animals have little if any chance of infection. In monkeys, however, tuberculosis is a more important problem.

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[10] **Nelson, J. B.** J. Exp. Med. **65:** 833–841; 843–849; 851–860. 1937.
[11] **Nelson, J. B.** J. Exp. Med. **72:** 645–654. 1940.
[12] **King, H. D.** Anat. Rec. **74:** 215–222. 1939.
[13] **Nelson, J. B., & J. W. Gowen.** J. Exp. Med. **54:** 629–636. 1931.

All species of monkeys and apes that are commonly used in laboratory work, as well as many that are not, are highly susceptible to tuberculosis. Moreover, conditions under which they are imported insure infection of a significant number.

Incidence of tuberculosis in newly imported monkeys varies from 10 to 25 per cent when large numbers are received in one shipment, and infection spreads readily, if the animals are confined in large groups in small cages. The route of infection is either respiratory or intestinal. Autopsy of cases that are allowed to run their course often fails to determine the portal of entry.

The average survival time of monkeys that have been infected naturally probably is about six months. The disease is not distinctive. The only reliable method of diagnosis during life is either one of two forms of the tuberculin tests.

The simplest tuberculin test for monkeys is the ophthalmic or palpebral test as developed by Schroeder.[14] This consists in a single injection of 1 milligram of Old Tuberculin or of the Purified Protein Derivative into the subcutaneous tissue of the upper eye-lid. Positive reactions appear between 48 and 72 hours. If the animal has tuberculosis, the eye-lid swells conspicuously. Otherwise, there is no response. This test is approximately 85 per cent accurate as determined by autopsy.[15]

The more complex tuberculin test, as devised by White and Fox,[16] is an adaptation of the original tuberculin test for cattle. It requires that the rectal temperature of each animal be taken at 3:00 P. M. for four days before subcutaneous injections of Old Tuberculin. On the morning of the fifth day, animals are injected and rectal temperatures taken at four-hour intervals for 48 hours. Abnormal temperature reactions indicate tuberculosis, but even a considerable experience in interpreting these reactions does not sustain one's self-assurance quite so well as does a monkey with a swollen eyelid.

The lesions of tuberculosis in monkeys are found mainly in the lungs, spleen and lymph nodes. In the lungs, one finds large and small areas of caseation necrosis, or actual pus formation. Sometimes, a whole lung is consolidated, and, on section, made up of alternate areas of caseous necrosis and darker, more vascular zones of recent involvement. Tracheo-bronchial lymph nodes are usually very large and necrotic except for a narrow rim of peripheral tissue. When infection enters by way of the intestinal tract, the mesenteric nodes show the major degree of enlargement. Tubercles in the spleen may vary from 0.1 to 1.0 centimeter, while in the liver the smaller size is the rule. In any of these lesions, tubercle bacilli usually are abundant and may be found in a smear without prolonged search.

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[14] **Schroeder, C. R.** Zoologica **23:** 397–400. 1938.
[15] **Kennard, M. A., & M. D. Willner.** Yale J. Biol. & Med. **13:** 795–812. 1941.
[16] **White, C. Y. & H. Fox.** Arch Int. Med. **4:** 517–528. 1909.

Control of tuberculosis in monkeys depends upon elimination of all reactors and exclusion of all sources of infection. By testing a colony at six-month intervals for 18 months or two years, and, thereafter, at yearly intervals, the disease may be eliminated, if, of course, personnel in charge of the animals is free of infection, and all new additions to the colony are free of disease.

6. **Miscellaneous Bacterial Disease.** Laboratory animals are subject to many other bacterial infections, some of which may occasionally cause epidemics. Rarely are these forms parasites of more than one species of animal. The more important of these will be discussed here.

"Mouse Septicemia,"[4] designates a relatively infrequent, slowly progressive disease caused by *Erysipelothrix muriseptica.* The chief manifestations of this infection are conjunctivitis, pneumonia, edema of abdominal tissues, enlargement of the spleen, and circumscribed small pale grey areas of necrosis in the liver. This organism of mice cannot be distinguished from *E. rhusiopathiae,* which causes swine erysipelas or "diamond-skin disease." Since mice are commonly employed as test animals for the diagnosis of swine erysipelas, there is a possibility of confusion. *Erysipelothrix* may be isolated by inoculating mice introperitoneally with emulsions of suspected lesions. If the material be infected, death follows in one to four days, the blood and spleen containing large numbers of the organism. It is a gram-positive, small slender rod, straight or slightly curved.

PYOGENIC INFECTIONS

Mice, rats and guinea pigs often develop abscesses of the subcutaneous tissues of the head, neck and other parts of the body. Often, the regional lymph nodes are also diseased. At times, the condition becomes frequent enough to be called epidemic especially among guinea pigs.[17] Streptococci, staphylococci, and other organisms have been isolated. Apparently, these bacteria gain entrance to the tissues through small surface wounds. The simplest method of dealing with this condition is to destroy affected animals and reduce contamination of the cages and animal houses.

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[17] **Megrail, E. & R. N. Hoyt.** J. Bact. **17:** 54. 1929.

In conjunction with these incidental diseases of small rodents, it may be well to consider briefly infection by *Streptobacillus moniliformis.* Strains of this organism are commonly found in the nasopharynx of rats. It is nonpathogenic for this species, but, in mice, it produces a highly fatal generalized infection. Essentially, this disease is characterized by multiple abscess formation in the joints and internal organs. At times, however, the infection may be a rapidly fatal septicemia. Other strains of this organism also cause abscesses in guinea pigs. The relationship of *S. moniliformis* to disease of rats, mice and guinea pigs is complicated by its association with organisms of the pleuro-pneumonia group. The pleuro-pneumonia organisms have been associated with polyarthritis of rats, with rolling disease of mice, and, in combination with *S. moniliformis,* as a cause of pyogenic infection of guinea pigs. Sabin's[18] monograph should be consulted for a complete discussion of these organisms.

PNEUMONIA

Epidemics of pneumonia caused by pneumococci and Friedlander's bacillus have been described in colonies of both guinea pigs and mice. In both species, mortality was high. However, such outbreaks of disease apparently are rare and occasioned by unusual circumstances. These animals ordinarily are not carriers of either type of organism.

In other instances, *Brucella bronchiseptica* has been isolated from epidemic pneumonia of mice and guinea pigs, and from pulmonary disease of rats. This organism has long been known as a contaminant of "snuffles" of rabbits, and distemper of dogs, and there is considerable doubt that it is naturally pathogenic for other animals.

BARTONELLOSIS

(Ref. 4, 5, 19, 20)

Organisms of the genus *Bartonella* are usually grouped with the bacteria, although their position is uncertain. Infections by these organisms occur widely in rats, mice, guinea pigs, and dogs, but become apparent only after splenectomy or some other serious disturbance of the immune mechanism. Then they apparently cause severe and sometimes fatal anemia. *Bartonella* is transmitted by blood-sucking arthropods, usually lice. Colonies free of lice are free of bartonellosis. Somewhat similar to *Bartonella,* are organisms assigned to the genera

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[18] **Sabin, A. B.** Bact. Rev. **5:** 1–16. 1941.
[19] **Weinman, D., & H. Pinkerton.** Ann. Trop. Med. and Parasitol. **32:** 215–217. 1938.
[20] **Groot, H.** Roc. Soc. Exp. Biol. and Med. **52:** 279. 1942.

Eperythrozoon and *Grahamella*. These are parasites of mice. Apparently, they are not pathogenic.

FUNGUS INFECTIONS

(Ref. 4, 5)

Fungus infections of laboratory animals usually are limited to the skin. They occur most often in dogs and cats—street animals that have many opportunities of contact. However, infections are well known in kennels, and spread easily. All of the rodents are susceptible and epidemics may occur among them. The common dermatotropic fungi are assigned to the genera *Microsporon* and *Trichophyton*. Lesions consist of small, circular, denuded, thickened, scaly patches distributed over much of the body, or larger, irregular, denuded areas that are reddened, moist and scaly. Spontaneous recovery sometimes occurs. It can be hastened by application of tincture of iodine or five per cent alcoholic solution of salicylic acid.

SPIROCHETAL INFECTIONS

(Ref. 3, 5)

Infections of laboratory animals by spirochetes are of little practical significance; but, perhaps, it may be well to note that natural infections occur both in dogs and rabbits. The incidence of *Leptospira icterohemorrhagiae* and *L. canicola,* organisms which cause jaundice and renal disease in dogs and Weil's disease in man, is approximately 20 per cent in this country.[21] This is something of a hazard to laboratory workers, but probably not a very great one. Spirochetosis of rabbits is a benign disease caused by *Treponema cuniculi* transmitted by contact, coitus, or inoculation.

VIRUS DISEASE

One is tempted to conclude that the virus infections of laboratory animals were designed more as a source of confusion than of morbidity. Under ordinary circumstances, few of these agents cause significant disease, but, when their hosts are inoculated with other agents or even stimulated by inert substances, the infection becomes apparent.

Probably, because of the great number of mice that are being used for experiment, a greater number of virus infections have been recognized in this than in any other animal.[22] Many of these are thoroughly discussed in Dingle's[4] review, but others have been reported more recently. I shall give only brief mention to these infections, listing them according to the chief regions of localization. One will, of course, realize that many viruses invade the body widely, although symptoms of infection may be related to one or another group of organs or tissues.

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[21] **Greene, M. R.** Am. J. Hyg. **34:** 87–90. 1941.
[22] **Thompson, J.** Arch. Path. **21:** 531–540. 1936.

Neurotropic Viruses: (1) Mice. Encephalomyelitis, Theiler;[23] flaccid paralysis of the hind legs, but not of the tail; incidence low, young animals chiefly. Meningoencephalomyelitis,[24] paralysis of hind legs; incidence unknown. Lymphocytic choriomeningitis:[25] Symptoms indefinite, young affected, morbidity and mortality low. Infected mice said to be more susceptible to intercurrent disease and lymphomatosis than other strains.[26] (2) Monkeys. Lymphocytic choriomeningitis[27] and "B" virus;[28] incidence unknown, inapparent infections until stimulated by other agents. (3) Guinea pigs. Ascending paralysis;[29] incidence unknown. May be caused by virus of salivary gland disease.[30]

Pneumotropic Viruses: (1) Mice. Virus pneumonia:[31, 32, 33, 34, 35] Many types, inapparent infections until stimulated by repeated passages. (2) Guinea pigs. Virus pneumonia:[36] Highly fatal disease, spread by contact; incidence unknown. (3) Hamsters:[37] (*Cricetus aruatus*) Virus pneumonia: Latent infectious until stimulated by repeated passage, incidence unknown. (4) Cats.[38, 39] Virus pneumonia: Highly infectious in young animals, mortality low.

Viscerotropic viruses: (1) Guinea pigs. Entero-hepatitis:[40] Acute disease, diarrhea, wasting; incidence unknown. Pneumonia-hepatitis:[41] A slowly progressive disease; incidence unknown. (2) Mice. *Infectious Ectromelia:*[4] Acute disease, often passing into chronic form. Acute state; polyserositis, and acute hepatitis: Chronic stage; edema, vesiculation and ulceration of skin of feet, and amputation of feet; incidence unknown, probably widespread, but usually inapparent.

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[23] **Theiler, M.** J. Exp. Med. **65:** 705–719. 1937.
[24] **Gahagan, L., & L. D. Stevenson.** J. Inf. Dis. **69:** 232–237. 1941.
[25] **Traub, E.** J. Exp. Med. **63:** 533–546. 1936.
[26] **Traub, E.** Zentralbl. Bakt. I. Abt. Orig. **147:** 16–25. 1941.
[27] **Armstrong, C., & R. D. Lillie.** U. S. Pub. Health Rep. **49:** 1019–1027. 1934.
[28] **Sabin, A. B., & A. M. Wright.** J. Exp. Med. **59:** 115–136. 1934.
[29] **Romer, P. H.** Deutsche Med. Wohnschr. **57:** 2685–2689. 1910.
[30] **Cole, R., & A. G. Kuttner.** J. Exp. Med. **44:** 855–873. 1926.
[31] **Horsfall, F. L., & R. G. Hahn.** J. Exp. Med. **71:** 391–408. 1940.
[32] **Herzberg, K., & W. Gross.** Zentralbl. Bakt. I. Abt. Orig. **146:** 129–239. 1940.
[33] **Freeman, G.** Proc. Soc. Exp. Biol. and Med. **48:** 568–610. 1941.
[34] **Nigg, C.** Science **95:** 49–50. 1942.
[35] **Karr, H. V.** J. Inf. Dis. **72:** 108–116. 1943.
[36] **Ten Broeck, C., & J. B. Nelson.** Proc. Soc. Exper. Biol. and Med. **39:** 572–575. 1938.
[37] **Pearson, H. E., & M. D. Eaton.** Proc. Soc. Exper. Biol. and Med. **45:** 677–679. 1940.
[38] **Baker, J. A.** Science **96:** 475. 1942.
[39] **Thomas, L. & E. M. Kolb.** Proc. Soc. Exper. Biol. and Med. **54:** 172–174 1943.
[40] **Marcos, Z.** J. Bact. **25:** 239–240. 1933.
[41] **Pappenheimer, A. M. & C. A. Slanetz.** J. Exper. Med. **76:** 299–306. 1942.

(3) Dogs. Distemper:[5] Acute generalized disease. Enteritis, or encephalitis may predominate; pneumonia secondary; world-wide distribution, highly contagious, often fatal; control by vaccination. (4) Cats. Panleukopenia:[42,43] Acute generalized disease, world wide distribution, highly contagious, often fatal; control by vaccination.

Epidermotropic Viruses: (1) Rabbits. Pox:[44,45] An acute disease, highly contagious, relatively uncommon, lymphadenitis, papules on skin and mucus membranes, keratitis, ophthalmia, orchitis; recovery prolonged, often fatal to young animals. "Virus III":[46] An inapparent wide-spread infection, produces skin lesions when transferred in series. Oral papillomatosis:[47] Common benign disease of buccal mucosa, transmitted by contact. (2) Dogs. Oral papillomatosis:[5] Highly contagious disease, spread by contact, causes serious inconvenience to the animal. (3) Canaries. Pox:[48,49,50] Highly fatal contagious disease, buccal muccosa, and viscera.

Salivary Gland Disease: An inapparent infection of guinea pigs, mice, rats, Chinese hamsters, and monkeys; inclusion bodies in hypertrophied cells of the ducts, of the salivary gland and sometimes kidneys.[51,52]

ANIMAL PARASITES

Coccidia, lice, and mites are the more important animal parasites of laboratory animals. One should be aware, however, that many other animal organisms, both protozoa and metazoa, may be found in the intestinal canal and solid organs of all species. Rats, mice and rabbits are readily infected by the larvae of dog and cat tapeworms. These parasites are introduced into animal colonies in bedding or food contaminated by dog and cat feces. Rats and mice, also, are readily infected by nematodes and cestodes, native to their wild relatives.[53] Street dogs and cats are commonly parasitized by several species of roundworms and cestodes. Monkeys, often, are infected by *Strogyloides, Oesophagostomum* and *Endamoeba histolytica.* With the exception of Coccidia and certain other protozoa, however, these parasites

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[42] **Hammon, W. D., & J. F. Enders.** J. Exper. Med. **69:** 327–352. 1939.

[43] **Kikuth, W., R. Goennert, & M. Schweickert.** Zentralbl. Bakt. I. Abt. Orig. **146:** 1–17. 1940.

[44] **Green, H. S. N.** J. Exper. Med. **60:** 427–440; 441–455. 1934.

[45] **Pearce, L., P. D. Roshan, & C. K. Hu.** J. Exper. Med. **63:** 241–258. 1936.

[46] **Rivers, T. M., & W. S. Tillet.** J. Exper. Med. **40:** 281–287. 1924.

[47] **Parsons, R. J., & J. G. Kidd.** J. Exper. Med. **77:** 232–250. 1943.

[48] **Shattock, S. G.** Lancet **1:** 1055. 1898.

[49] **Kikuth, W., & H. Gollub.** Zentralbl. Bakt. I. Abt. Orig. **125:** 313–320. 1932.

[50] **Burnet, F. M.** J. Path. and Bact. **37:** 107–122. 1933.

[51] **Kuttner, A. G., & S. Wang.** J. Exper. Med. **60:** 773–791. 1934.

[52] **Thompson, J.** J. Inf. Dis. **58:** 59–63. 1936.

[53] **Hall, M. C.** Proc. U. S. Nat. Mus. **50:** 1–258. 1916.

either may be eliminated easily by treatment or their invasion of the colony, prevented completely by application of simple hygienic measures.

PROTOZOAN INFECTIONS

Coccidiosis: [54] As a rule, coccidiosis is a disease of young, growing animals. Among the laboratory animals, its severest forms occur most commonly in rabbits; but, under unusual circumstances, other animals may be seriously affected.

Coccidia present striking examples of host specificity and, sometimes, of organ specificity. Rarely are species of these protozoa interchangeable between different animal species. Thus, control is not so complex as it is with some of the pathogenic bacteria; but, even so, factors determining the spread of the infection and its severity remain unchanged. That is: the parasite is maintained by carriers, it is spread by contact and, on the whole, severity of disease is influenced both by the size of the infecting dose and the susceptibility of the animal.

Coccidiosis of rabbits involves both the liver and the intestines. Either or both forms of the disease may occur in the same host. One is no protection against the other, and both types may vary from rapidly fatal infections to mild inapparent disease. In either hepatic or intestinal coccidiosis, severe disease is accompanied by diarrhea and wasting, and subacute or chronic cases of hepatic coccidiosis are accompanied by marked abdominal enlargement.

Post mortem examination of animals dying of hepatic coccidiosis finds the liver, gall bladder and bile ducts hypertrophied; and, if the disease has been subacute or chronic, many white or yellow nodules, up to two centimeters in diameter, are scattered over the liver. Depending upon their age, the contents of these lesions vary from thin whitish fluid to thick caseous material, which later may become calcified and mistaken for tubercles. In animals dying of intestinal coccidiosis, the small intestine is dilated and pale, and the mucosa may be thickened.

Hepatic coccidiosis of rabbits is caused by *Eimeria stiedae;* intestional coccidiosis by *E. perforans, E. magna* and possibly other species. Infections may be recognized before death by finding the characteristic oocysts in the stools. Identification of species requires careful measurement. At autopsy, oocysts may be found in large numbers in the early nodules of the liver, but are more difficult to identify from the intestinal epithelium.

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[54] **Becker, E. R.** Coccidia and Coccidiosis of Domesticated Game and Laboratory Animals and of Man. Collegiate Press, Inc., Ames, Iowa. 1934.

Control of coccidiosis is difficult, because the parasite is so widely distributed and is transmitted from mother to offspring so readily. Modern hutches which prevent fecal contamination of food and the cage, or at least reduce it to a minimum, do much to prevent losses from this disease.

Dogs, cats, rats, mice, and guinea pigs, also, are parasitized by one or more species of coccidia; but, on the whole, these animals are not often seriously affected by the organisms. If, however, they are fed large doses of oocysts, dogs, rats and guinea pigs may develop severe enteritis.

Other Protozoan Infections: In addition to coccidia, many other species of protozoa occur regularly in the intestines of rodents, rabbits and monkeys. With the exception of *Endamoeba histolytica,* a common parasite of many species of monkeys and rarely of dogs, none of these organisms cause disease, and the importance of *E. histolytica* may be questioned. I have seen a large number of monkeys that carried *E. histolytica* without evidence of disease either before or after death, and have seen it cause disease only in animals of the genera *Ateles* and *Lagothrix.*

Protozoan parasites of the blood and tissues also may occur among laboratory animals. Sometimes, they may cause disease, but possibly more often, they cause confusion. One of the most common is *Toxoplasma gondii*[5, 55, 56] an organism of undetermined position, but with a wide range of hosts. It has been found in mice, rats, guinea pigs, rabbits, dogs, cats, sheep, man and many species of birds. In some instances—rabbits, and man—it has been associated with encephalitis, but again it has been found only when brain tissue was inoculated into other animals. The infection in cats and dogs involves tissues other than the brain.

Somewhat similar to *Toxoplasma* are the Sarcosporidia. These are parasites of muscles of a variety of animals. In the muscles, one may find tiny white tubular structures visible to the unaided eye or, again, the parasite is found by chance in section of muscle as a thin-walled tube containing bodies three to seven microns in length. Apparently, these organisms do not cause active disease.

It also may be well to mention *Babesia canis,* a parasite of dogs throughout the warmer parts of the world. This organism is trans-

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[55] **Sabin, A. B., & P. K. Olitsky.** Science **85:** 336–338. 1937.
[56] **Wolfe, A., D. Cowen, & B. H. Page.** Amer. J. Path. **15:** 657–694. 1939.

mitted by several species of ticks, and animals that are exposed early in life usually suffer little, but in non-immune dogs, there is acute disease, with high fever, anemia, jaundice and hemoglobinuria. Thus, the disease simulates leptospirosis. *B. canis* is a parasite of erythrocytes and is easily transmitted by transfusion.

METAZOAN PARASITES

The cestodes and nematodes that parasitize laboratory animals are not particularly significant. However, it may be well to recall that the hookworms of dogs are widely distributed and, since they are blood-sucking organisms, may interfere with experiments. Dogs may be infected by any one of three species of hookworms: *Ancylostoma caninum, A. braziliense,* and *Uncinaria stenocephala.* Infections are recognized by finding characteristic ova in the stool. Stool examination is greatly facilitated by concentration or flotation methods.[57]

Strongyloides stercoralis, another nematode parasite of the intestine, may occur in dogs, cats and monkeys and be associated with diarrhea. The larvae of this species are found in the feces. Monkeys are also parasitized by species of *Oesophagostomum,* which produces inflammatory nodules in the wall of the colon and may cause death by perforation of one of these lesions. This parasite is rapidly lost if reinfection be prevented by reducing fecal contamination of the food.

Acarasis: All species of laboratory animals may be infected by mites, some of which merely hide in the cages and take blood at night, but others burrow into the skin of the head, ears and body causing considerable damage to their hosts. *Sarcoptes scabiei* causes one form of mange in dogs, *Demodex canis* another, and species of *Otodectes* infect the ears of dogs, cats and occasionally rabbits; but *Psoroptes cuniculi* is the more common cause of ear mange in rabbits. However, there is little profit simply in listing the names of the various species of mites and lice that may be found on laboratory animals. Lesions produced by the mange mites are all more or less similar. The skin is bare, thickened and covered by crusts. The parasites may be found by scraping the lesions deeply, emulsifying the crusts in water or sodium hydroxide solution, and examining them under the microscope, or the larger mites may be seen with the hand lens or unaided eye. Identifications may be made by reference to any good text, such as Mönnig's "Veterinary Helminthology and Entomology."[58]

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[57] **Coffin, D. L.** Manual of Veterinary Clinical Pathology. Comstock Publishing Co., Inc., Ithaca, N. Y. In Press.

Control of mites that do not invade the skin may be accomplished by spraying the cages with light volatile oils, or by sterilizing cages with steam or dry heat. Ridding animals of mange and follicle mites is considerably more of a problem and requires careful treatment of all affected animals at intervals of 7 to 10 days, as well as disinfection of cages, and animal rooms. Mönnig lists formulae that have proven useful in treatment.

In attempting this review, a major problem has been the choice of material to be included. An all-inclusive survey obviously would be impossible. A second question has been the space allowance for any one disease. An adequate consideration of the infectious diseases of laboratory animals, with illustrations of morbid processes and directions for diagnosis and control, would occupy a volume of several hundred pages. Much of this would merely be repetition of readily available information. Possibly, such a volume might be valuable to some, but the effort would be difficult to justify. The present survey can be regarded only as an introduction.

DISCUSSION OF THE PAPER

Dr. C. A. Slanetz (*College of Physicians and Surgeons, Columbia University, New York, N. Y.*):

Dr. Ratcliffe has reviewed the important infectious diseases of laboratory animals so adequately that I can add little except some of our experiences with disease control of small animal colonies at the College of Physicians and Surgeons Columbia-Presbyterian Medical Center. Our observations cover a period of 14 years. Although, for the most part, they fit the picture as presented by Dr. Ratcliffe, we have made, I believe, some observations which are different and which may be of interest to individuals concerned with the origin and care of experimental animals.

As indicated by Dr. Ratcliffe in his report, we also have found *Salmonella* organisms to be the most important cause of disease among mice, rats and guinea pigs. The success we have had in maintaining breeding colonies of rats, guinea pigs and mice practically free of paratyphoid infections, for the past ten years, has been very helpful in our work at the college. Our program of disease control includes testing of breeders at monthly intervals and, at times, more frequently. Fecal specimens are taken and streaked on brilliant green agar, containing a dye dilution of 1:260,000. No difficulty has been encountered in keeping our rat colony entirely free of infection.

With mice, the problem has been less simple. Occasionally, it has been necessary to culture the breeders more frequently than once a month to make sure that no paratyphoid carriers remain in the colony. *S. enteritidis* and *S. typhimurium* are the two species which we have found in most instances.

Our experience in large scale testing includes a commercial mouse colony which supplies a part of our needs. The results have been encouraging. At present, the incidence of *Salmonella* infection in that colony is a fraction of one per cent, judging from tests on groups of 200 to 400 mice, compared to an average of 15 per cent before testing, was instigated five years ago. Stock mice from several commercial sources have shown an incidence of 5 to 30 per cent paratyphoid carriers.

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[58] **Mönnig, H. O.** Veterinary Helminthology and Entomology. 2nd. ed. Williams and Wilkins Co., Baltimore. 1941.

So far, our guinea pig colony has not given us any difficulty with paratyphoid, even during the breeding period when others report *Salmonella* infections to be prevalent in pregnant females and in females at parturition.

We have not encountered paratyphoid in our rabbit breeding stock.

Control of rabbit coccidiosis has been difficult. By frequent examination of fecal specimens (flotation method) of breeding stock, selecting negative does, and the use of screen floors in cages, we had been able to reduce the incidence of liver coccidiosis in young stock from 90 per cent to approximately 5 per cent. The cost of such a program is considerable and is not practical on a large scale.

When an epidemic of snuffles occurs among our rabbits, the survivors usually prove resistant to snuffles in subsequent epidemics.

Tuberculosis in monkeys is no longer a problem with us. By housing healthy stock in individual metal cages with screen floors, solid sides, and proper care of food, we have been able to maintain a colony of 40–60 monkeys over a period of 8 years with only three deaths from tuberculosis. When we formerly housed monkeys in groups of 6–10, our losses from tuberculosis were high at the end of 6–8 months.

Dog distemper is still a disturbing factor in our dog experimental program. Our dogs are purchased from a dealer in Pennsylvania and are probably exposed to distemper at the kennels there or in contact with distemper cases in the shipping crates. By injecting small dogs and puppies with suitable doses of anticanine distemper serum followed by the regular prophylactic vaccination we have been able to develop immunity in such animals. Large breeds of dogs, shipped to us from Pennsylvania, have not always responded satisfactorily to such treatment, possibly because of inadequate doses of serum or other factors.

Dr. C. R. Schroeder (*Lederle Laboratories, Pearl River, New York*):

The infectious and parasitic diseases of laboratory animals have been ably and amply covered by Dr. Ratcliffe.

Biological and pharmaceutical manufacturing plants are probably the largest users of small animals today for testing and experimental purposes. The infectious disease problem has great economic importance for this group. Hemolytic streptococcal adenitis and salmonellosis are the cause of greatest losses.

Specifically immune animals cannot be used because the common group of test animals must have equal susceptibility to all the genera of organisms, toxins and viruses to which they may be exposed.

The maintenance of large, confined colonies of laboratory test animals with rapid population turnover, free of interfering infectious disease, is a difficult management problem.

Control measures include:

1. Systematic testing to screen out carriers and prevent the introduction of infectious disease and parasites. Maintaining adequate quarantine and only admitting clean stock to breeding colonies and testing units.
2. Furnishing proper housing, including:
   a. adequate temperature and humidity control;
   b. avoiding over-crowding;
   c. furnishing suitable bedding;
   d. avoiding draughts and excessive noise.
3. Maintaining adequate diets.
4. Giving attention to good sanitary practices, including:
   a. proper disposition of sick and dead animals and waste;
   b. thorough cleaning and disinfection of buildings and cages.

Sanitation, including the use of mechanical cleaners, cleaning compounds and disinfectants, has not been discussed and it seems timely and in order to treat it here.

There are many types of cleaning units which will physically remove organic matter from any surface, regardless of its composition.

Three types of equipment are available:

1. Flash boiler steam generators:
   Steam pressure, in excess of 100 pounds, is generated to propel water and heat. An injector forces water and detergent in correct proportion into the steam line, so that an ample stream of water with detergent at 100° C. in excess of 100 pounds pressure is delivered to the nozzle at the end of a steam hose, to be directed at close proximity to the part to be cleaned.

   This type of unit may be stationary or mounted on a trailer to permit delivery to the job where plant steam is not available. A source of water, together with a 110 volt outlet, is needed. The boiler fuel is kerosene or the equivalent. (This equipment is distributed by the Homestead Valve Manufacturing Company, Coraopolis, Pennsylvania.)
2. A unit composed of pumps which build extremely high cold water pressures in excess of 400 pounds in 10 seconds (manufactured and distributed by the Drill Manufacturing Company, Circle Tower, Indianapolis, Indiana).
3. Units for steam cleaning, utilizing available plant steam of 75 to 150 pounds pressure. These units syphon a premixed solution from a portable reservoir and inject it into the steam line (manufactured and distributed by the Kerrick-Hydro Steam Kleaner, Clayton Manufacturing Company, Alhambra, California, and Oakite Products Inc., 46 Thames Street, New York City).

The following manufacturers may be consulted in selecting the proper detergent to be used in conjunction with the above equipment for efficient cleaning.

Dextrex Corporation, 13013 Hillview Avenue, Detroit, Michigan; Kemico Mfg. Company, Irvington, New Jersey; Oakite Products, Inc., 46 Thames Street, New York City; Turco Products, 48th and South Holsted, Chicago, Illinois; U. S. Sanitary Specialties Corporation, 437 South Western Avenue, Chicago, Illinois; Wyandotte Chemicals Corporation, Biddle Avenue, Wyandotte, Michigan.

Final disinfection can be accomplished by using any of the quarternary ammonium compounds like Roccal. Chlorine compounds such as H T H, perchloron, or chlorinated lime, may be used, but they are destructive to metal parts. Mercurial and coal tar preparations should be avoided. Usually, thorough cleaning with ample water and detergents precludes the necessity of a complex disinfection program.

INFLUENCE OF ENVIRONMENTAL TEMPERATURES ON WARM-BLOODED ANIMALS

By Clarence A. Mills

From the Department of Experimental Medicine, University of Cincinnati, Ohio.

Studies of recent years have gradually exposed the dominance which environmental temperatures exert over the existence of all warm-blooded animals. Ease of body-heat loss is now seen to play a major role in determining such vital factors as growth rate, speed of development, fertility, resistance to infection, dietary requirements for certain vitamins, and even mental ability. In order to understand just why and how this should be so, it is necessary to consider the animal body as a combustion machine.

Dynamics of warm-blooded existence. All body functions require energy for their performance, and the only source for such energy is the cellular combustion of foodstuffs—chiefly glucose. Unfortunately, various studies have shown homothermic animals and man to work at relatively low efficiency, being able to transform only 20–25% of their combustion energy over into work output. This is similar to the working efficiency of a good gasoline motor but far below the 37+% working efficiency now achieved by the Diesel engine.

This low efficiency of the animal body means that 75–80% of all combustion energy must be dissipated as waste heat; and, since heat accumulation (fever) seriously disturbs many important functions, it is necessary that this dissipation be readily accomplished. Hence it is that environmental temperatures—controlling, as they do, the ease or difficulty of body-heat loss—assume a truly dominant role in the existence of warm-blooded animals and man.

Variations in metabolic rate. Figure 1 shows variations in the basal metabolic rate of man which take place in southern Germany (at Heidelberg), as outdoor mean temperatures rise and fall through the seasons. Gelineo[1] has demonstrated a similar metabolic responsiveness in experimental animals under controlled conditions, as has also Lee.[2] These changes in basal or resting metabolism require 2 to 3 weeks for their appearance after environmetal temperatures have risen or fallen. The more immediate response to temperature change involves skeletal muscle tone with relaxation in heat and greater muscular activity or shivering in cold.

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[1] **Gelineo, S.** "Influence du milieu thermique d'adaptation sur la thermogénèse des homeothermes." Ann. de Physiol. **10:** 1083. 1934.

[2] **Lee, Robert C.** Heat production of the rabbit at 28° C. as affected by previous adaptation to temperatures between 10° and 31° C. J. Nutr. **23:** 83. 1942.

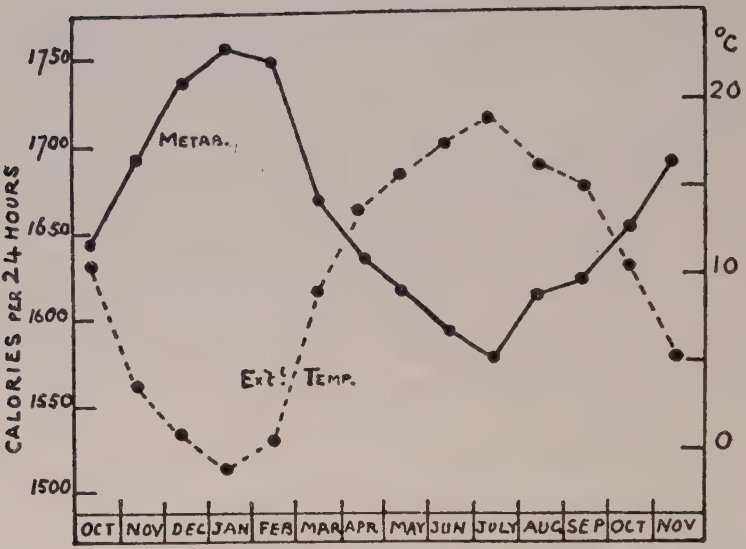

FIGURE 1. Mean monthly metabolism and mean monthly temperature. Gessler (1925), observations on himself.

The slower changes in basal metabolism should be kept constantly in mind. They bring profound alterations in nutritional requirements and the functions of many organs in animals grown or kept for experimental studies. Temperature control in animal quarters is now recognized as a necessary factor in careful research.

Growth and development. FIGURE 2 illustrates the typical growth retardation brought by tropical or summer heat, with mice receiving Purina chow *ad libitum.* No difference is seen during the first week of heat exposure, but, beginning in the second week and continuing on indefinitely, there occurs a 40% growth deficit in the heat. Only about half as much is eaten by the hot-room animals, so their metabolic efficiency is actually greater, giving more weight gain for each gram of food eaten. After 2–3 months, mice and rats in the heat are found to have tails considerably longer than those of their litter mates in the cold. Rabbits grow distinctly larger ears to facilitate heat loss when environmental temperatures are kept high. These developmental adaptations in heat-loss organs reflect quite clearly the metabolic handicap such warm-blooded animals face in hot surroundings. Within 2–3 weeks after heat-adapted animals are removed to cool surroundings, they resume a normal food consumption and growth rate.[3]

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[3] **Mills, C. A.** Medical Climatology. C. C. Thomas, Springfield, Mass. 1939.

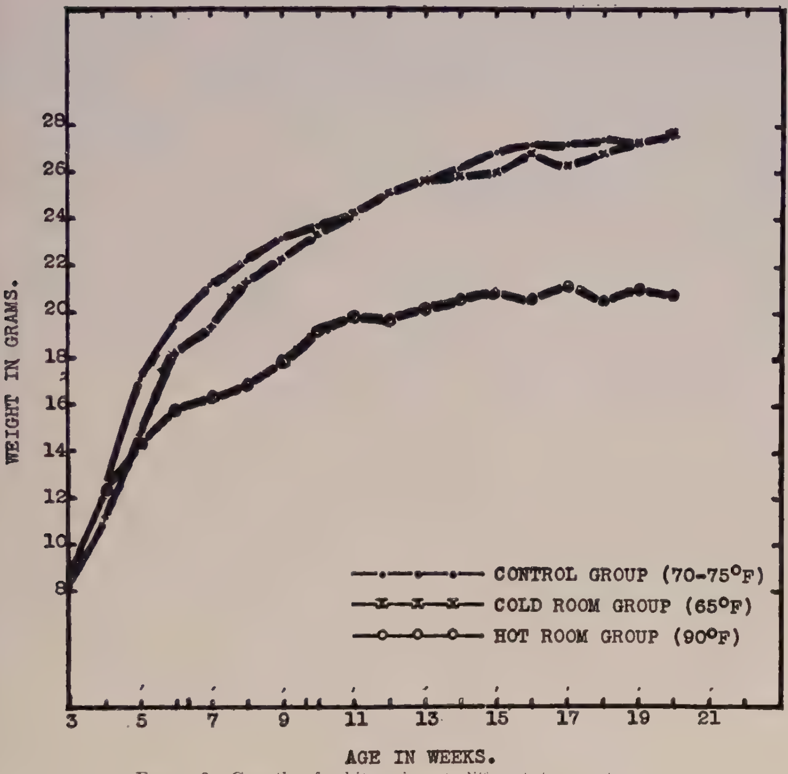

FIGURE 2. Growth of white mice at different temperatures.

Onset of sexual functions and fertility. Mice kept at 90–91° F., from weaning age on, begin their sexual cycles significantly later than litter mates kept in cool quarters. Fertility in both males and females also comes on later in the heat and is, at all times, at a lower level of effectiveness. Mating takes place just as readily in hot as in cool environments, but conceptions are difficult to achieve in the heat, while almost every mating is effective in the cold. Litter size is significantly larger at 70° F. than at 90° F., with fewer still-births or deaths during the first week of life. Second-generation mice in the heat are almost completely infertile. Transfer from heat to cold usually results in a restoration of normal fertility within 3 weeks. Over-heating, with hyper-pyrexia for some hours, may result in permanent loss of fertility, even though the animal appears to be in perfect health otherwise.

Ability to store and mobilize glycogen. Liver glycogen is usually found to be less in heat-adapted animals, but more striking is their inability to mobilize the stores they do have when exposed to body chilling. Those kept in cool quarters exhaust their liver glycogen almost completely in an effort to maintain a normal body temperature under chilling conditions, but those adapted to heat suffer a severe fall in rectal temperature and are prostrated by the cold, without having used more than a small fraction of their stored glycogen. This inability to meet chilling renders heat-adapted animals very susceptible to pneumonia when exposed to varying temperatures.

Mental Functions. While most of us are not often concerned with the thinking capacity of our laboratory animals, there are certain studies which deal directly with this phase of physiology.[4] It is thus important to know that environmental temperatures and ease of heat loss markedly affect mental functions. Rats adapted to different temperatures (55° F., 75° F. and 92° F.) for 3 months were tested for their ability to learn their way through a maze to food, after a 24 hours fast. Those kept at 55° F. required 12 trials and made few mistakes subsequently; those from the 75° F. quarters learned only after 28 trials and still made many mistakes; many of the rats adapted to 92° F. never did find the way to the food, while the successful ones found the food only after an average of 53 trials! Retested again, after a month's rest, those from the 55° F. room were found to have perfect retention of their previous learning, those from the 75° F. room had to relearn about half, while the previously successful ones from the heat showed no evidence whatever of their former experience. Thus, the ability to acquire and retain learning seems sharply dependent upon the ease of body-heat loss and the metabolic level allowed the animal. I might say that college students show similar heat effects!

ENVIRONMENTAL TEMPERATURES AND NEOPLASTIC TENDENCIES

Underfeeding, with the resultant malnutrition, has been shown to reduce the incidence of spontaneous tumors and leukemia in susceptible mice. Environmental warmth exerts a similar effect with mice on a Purina chow diet, perhaps because of a like lowering of food consumption and growth retardation. Both dba and C_3H strain mice show a significant drop in spontaneous breast tumor incidence, if raised at 90° F., as compared to 70° F.[5,6] The tumors occur at an earlier age, grow in size faster, and are more likely to be multiple in the mice adapted to stimulating coolness. Spayed females, while showing a marked reduction in breast-tumor incidence, still exhibit this same stimulating effect of a cool environment.

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[4] **Hellmer, Leo A.** The effect of temperature on the behavior of the white rat. Am. J. Psychology, **56:** 408–421. 1943.

[5] **Wallace, Edward W., Helene Wallace, & C. A. Mills.** Influence of environmental temperature upon the incidence and course of spontaneous tumors in C_3H mice. Cancer Research. **4:** 5. May, 1944.

[6] **Fuller, R. H., Edna Brown, & C. A. Mills.** Environmental temperatures and spontaneous tumors in mice. Cancer Research. **1:** 130–133. 1941.

With chemically induced or transplanted tumors the story is quite different.[7] If methyl-cholanthrene be injected subcutaneously into C_3H mice, those previously adapted to 90° F. show a higher incidence of tumor development, earlier appearance and more rapid growth of the mass, and an earlier death of the animal. An even more striking difference occurs with subcutaneous transplantation of tumor cells. In the heat, the incidence of "takes" is very high and growth of the mass rapid, while, in the cold, most implantations show only a slight period of growth before their regression and complete disappearance. With implantation into thigh muscles, however, there is noted no significant difference between hot- and cold-room tumor transplants.

These findings lead naturally to the conclusion that the level of tissue metabolism or richness of blood supply is probably an important factor in neoplasia. In hot surroundings, the rich blood supply to the skin for heat-loss purposes results in an active cutaneous metabolism and increased tendency to neoplasia. In cool environments and low skin temperatures, skin tumors are induced with greater difficulty, but spontaneous tumor incidence is higher in the deeper tissues and organs which have a more active metabolism in the cold. Whatever the mechanism by which the differences are produced, it is obvious that temperature control should be exercised in all tumor studies.

Resistance to infection. Animals adapted to 90° F. are much more likely to develop pneumonia, when chilled, than are those adapted to 70° F. Mice on Purina chow diet in the heat die significantly earlier after inoculation with a fixed number of Type I pneumococci than do those kept in the cold. With a less virulent organism (*Streptococcus*), dosages can be found which kill all heat-adapted mice, but fail to kill those from cool quarters. This lowered resistance in the heat is accompanied by only a slight reduction in ability to produce immune bodies or in the amount of immune serum required for passive protection.[8] It would therefore seem likely to be based upon differences in phagocytic activity in the heat and cold. Studies along this line will be mentioned in a later section dealing with nutritional requirements.

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[7] **Wallace, E. W., Helene Wallace, & C. A. Mills.** Effect of climatic environment upon the genesis of subcutaneous tumors induced by methylcholanthrene and upon the growth of a transplantable sarcoma in C_3H mice. J. Nat. Cancer Inst. **3:** 99–110. 1942.

[8] **Mills, C. A., & L. H. Schmidt.** Environmental temperatures and resistance to infection. Amer. J. Tropical Medicine. **22:** 6. Nov. 1942.

Longevity. If the slower-growing mice in hot environments be carefully protected from chilling and infectious epidemics, they exhibit the same increased longevity that McKay obtained in his experiments on underfeeding. Voluntary underfeeding from loss of appetite and difficult heat loss in hot environments is probably quite analogous to the enforced malnutrition brought by limitation of food supply at ordinary temperatures.

Drug toxicity. Animals adapted to hot environments are hypersusceptible to the action of such drugs as insulin and thyroid extract.[9] The convulsive dose of insulin for mice is only 1/20–1/30, as much when they have been adapted to 90° F. as in cool surroundings (68° F.). With thyroid extract in rats, the difference is about 10-fold. Animals in the heat are, in general, much more susceptible to convulsant drugs such as strychnine and picrotoxin. Some of the sulfonamides have also been found more toxic in the heat. More studies are needed along this line.

Nutritional requirements. In an effort to counteract or prevent these unfavorable heat effects, we have made an extensive investigation of possible differences in nutritional requirements. First attention was centered upon the various B-vitamins, several of which are known to play essential roles in cellular combustion. It seemed possible that dietary enrichment with these combustion catalysts might make for greater metabolic efficiency, allowing the animal a higher level of existence in the continuing presence of difficulty in heat loss.

Briefly stated, it has been found that best growth is obtained in rats and mice kept at 90° F., when the dietary *thiamine* is twice as high (per gram of food) as is needed for optimal growth at 70° F.[10] For young rats, this means 0.8 mg. per kilogram of food at 70° F. and 1.6 mg. per kilogram at 90° F.; while, for mice, analogous requirements are about 2 mg. per kilogram and 4 mg. per kilogram. With advancing age, rats at 70° F. show no change in requirement, but those kept at 90° F. exhibit a progressive rise (to as much as 3 mg. per kilogram by 9 months of age). These differences in thiamine requirement, per gram of food in heat and cold, are not due to suppression of thiamine synthesis by intestinal bacteria in the heat, for they persist unchanged when 0.5% sulfaguanidine is added to the diets.

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[9] **Chen, K. K., Robert C. Anderson, Frank A. Steldt, & C. A. Mills.** Environmental temperature and drug action in mice. J. Pharmacol. and Exper. Therapeutics **79:** 2. Oct. 1943.

[10] **Mills, C. A.** Environmental temperatures and thiamine requirements. Amer. J. Physiology **133:** 3. July 1941.

Choline is also required in larger amounts in hot-room diets if optimal growth and development are to be attained. Rats at 70° F. do well on as little as 0.5 gram of choline per kilogram of diet—in fact, they grow normally on a choline-free diet after the danger period for acute hemorrhagic nephritis has been passed. In the heat, however, rate of growth is directly related to the amount of dietary choline, being optimal only at 5 grams per kilogram of food. This substance thus becomes an important growth factor in hot environments.[11]

On a Purina chow diet, growth of rats and mice exhibits a 40% retardation in the heat. This handicap is reduced by half when the hot-room diets are properly enriched with thiamine. Further growth improvement with choline addition leaves only a 5–10% growth deficit in the heat. This remaining handicap can be completely eradicated by raising the protein content of the hot-room diets from 18% to 24% (casein) or by the addition of 0.2% cystine.

After the indicated thiamine, choline, and protein corrections have been made, growth and development in the heat are identical with those seen in cool surroundings. Ear and tail overgrowth no longer takes place in the heat, indicating the likelihood of a greater metabolic efficiency.[12] Body phagocytes from heat-adapted animals are just as active in ingesting bacteria as are those from animals kept in the cold, provided the higher nutritional requirements of the heat have been met.[13] No study has yet been made to see whether such dietary corrections would eliminate the differences in neoplastic tendencies shown by mice of susceptible strains in heat and cold. Nor have experiments been completed to show whether these corrections would make resistance to infection the same at all environmental temperatures.

GENERAL DEDUCTIONS

It now seems obvious that temperature control in animal environments is essential if experimental results are to be uniform and reproducible. This is particularly true where warm-blooded animals are to be used for drug standardization tests, for vitamin assays, or for the determination of nutritional requirements. It is also true in any studies on animal psychology or mental behavior patterns. This factor should be carefully controlled in all studies on resistance to infection, red cell and hemoglobin production, and phagocytic functions. And, of course, it is of paramount importance for the maintenance of optimal fertility in breeding stock and of high vitality and rapid growth of the young.

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[11] **Mills, C. A.** Environmental temperatures and B-vitamin requirements. Archives of Biochemistry **1:** 1. Oct. 1942.

[12] **Mills, C. A.** The B-vitamins in tropical nutrition. Internat. J. Leprosy **10:** 82. 1942.

[13] **Cottingham, Esther, & C. A. Mills.** Influence of environmental temperature and vitamin-deficiency upon phagocytic functions. J. Immun. **47:** 6. Dec. 1943.

When it is found necessary to grow or use experimental animals in summer or tropical heat without artificial cooling, proper fortification of the diet should be made to meet the heightened requirements. These have been indicated for the rat and are now being determined for the chick. No such studies have been made for guinea pigs, rabbits, dogs or other experimental animals.

Domestic livestock show very clearly the retarding effects of tropical heat, for steers on the best ranches of tropical lowlands take 4–5 years to reach the 1,000-pound slaughter size, whereas this size is attained in 1½ years in our northern states. Hogs in Panama require 15 months to reach the 200-pound size achieved in 6–7 months in cooler lands. Cows of excellent stock, imported from the United States into the Canal Zone and fed the best known food mixtures, produce 25% less milk than they did in cooler climates.[14] The matter is a very important one in animal husbandry and will repay the animal grower for his careful attention to the subject.

Best temperatures for breeding rooms containing suckling young are in the neighborhood of 76–78° F., but the breeders, before delivery, will have highest vitality to transmit to the offspring if kept at temperatures of 68–72° F.; and the young, after weaning, will also have highest vitality at these temperatures.

Uniformity of temperature is of great importance, at all times, in the prevention of pneumonic infections. One series of cancer mice was observed over a 20-month period, under 3 different temperature conditions: 68° F., 90° F., and in the variable temperatures of an ordinary laboratory room. Among 66 mice kept in the air-conditioned room at 90° F., none of those dying showed evidences of pneumonia at autopsy. In the room kept at 68° F., there was one such, but among the 66 exposed to the more variable laboratory conditions, there were 13 with evidence of pneumonia at death.

It will pay animal growers to keep in mind, at all times, that proper ease of body-heat loss is a dominant factor in warm-blooded individuals of any species, that such individuals are combustion machines of relatively low working efficiency, needing quick dissipation of waste heat from the body. Difficulty in such dissipation, if prolonged for

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[14] **Mills, C. A.** Climate Makes the Man. Harper Bros., New York. 1942.

2–3 weeks below the fever-producing level, results in a more sluggish cellular metabolism, a general lowering of vitality, and a drop of fertility.

DISCUSSION OF THE PAPER

In answer to various questions, **Dr. Mills** made the following comments:

The food factor is a very important one, of course. Corn-fed steers mature faster than those ranging on the best pastures. Alfalfa is rich in the B-vitamins and greatly accelerates growth when mixed with grain-feeding. On tropical ranches, the grass is of poor quality, reedy and tough, and the steers get no grain. This inadequate diet undoubtedly plays a large part in the retarded development seen there. But we now know that there would be some retardation by the heat, even with the best feeding methods, unless artificial supplementation of the diet is carried out.

Animals in the cold can tolerate the doubled dietary thiamine needed by those in the heat, but they do not do well with the 6-fold increase in choline needed by the hot-room animals.

Oxygen consumption studies should be carried out at a uniform neutral temperature. 28° C. is commonly used and is considered to be the point of thermal neutrality for most warm-blooded animals.

Obviously, oxygen consumption tests carried out at 32° C. could not be compared with those made at 20° C., for the immediately prevailing rate of body-heat loss in the cold would bring on a direct combustion increase (largely through increased skeletal muscle tone). The effect of climate, or of long-continued heat or cold, is of slow onset, requiring about three weeks for its full development. Three weeks of tropical moist heat depresses the metabolism of animals and this depression is still in evidence after a day spent at 28° C. Long-continued cold likewise brings on a metabolic stimulation which persists for several days when the animal is placed at 28° C. It is this slower and more lasting metabolic effect through which climate exercises its chief influence.

In the drug-toxicity studies described in the paper, the animals were adapted for only one week before the injections were made. This work should be repeated with the full 3-week period for adaptation.

Even with the most perfect food mixtures so far devised, milk production at the Minde Dairy in the Canal Zone is 25 per cent less than was yielded by the same cattle before being shipped down to the Zone.

ANIMAL COLONY MAINTENANCE—FINANCING AND BUDGETING—VIEWPOINT OF THE UNIVERSITY

By Sidney Farber

Harvard Medical School, Boston, Mass.

The extraordinary advances in the biologic sciences during the past fifty years have been based in great part upon data obtained from the use of laboratory animals. It is recognized, too, that a large proportion of the important progress in human and veterinary medicine would have been impossible without the opportunity for unhampered animal experimentation. The universities and, specifically, the medical and allied schools, therefore, have a real interest in the problems of animal colony maintenance and in a discussion of such considerations of importance to the raising of animals, as genetic purity, nutrition, environmental conditions, and infectious disease. A total of many thousands of mice, rats, guinea pigs, rabbits, cats, dogs, monkeys, hogs, fowl, frogs, turtles and leeches are used each year in the teaching of students and in the conduct of research at Harvard Medical School, the Public Health School, and the School of Dental Medicine. The conditions which govern the care and use of these animals are the responsibility of an animal committee composed of faculty members appointed annually by the President. It may be stated here that all teaching exercises and experiments, involving the use of animals, must be conducted according to regulations set, and under the possibility of unannounced inspection by this committee. Copies of the regulations are displayed prominently in all animal rooms of the school. They guarantee humane and intelligent handling of animals. They are uniform with those adopted by the American Medical Association. For the efficient and economic purchase and delivery of so many animals of such different kinds, the school maintains a central Animal House. This is a self-contained one-story building structurally attached to the school building. It contains a series of rooms designed to hold cages for animals and tanks for turtles and frogs, a feed room, a utility room, a kitchen with facilities for the preparation of foods of various kinds, a large refrigerator and an incinerator. The Animal House is cared for by two full-time men—the manager and his assistant—who order, receive and deliver animals; prepare food, clean cages, care for animals and, in general, keep the quarters in a satisfactory condition. The part-time services of a secretary in the Dean's office are required for the bookkeeping. All financial transactions are controlled by the Bursar's Office at Harvard University.

The Animal House is, in effect, a retail store for the sale of animals, and of foods such as horse meat, to the various departments of the school. Individual research workers or department heads may deal directly with commercial firms for the purchase of animals without the intervention of the Animal House. Years of experience with this retail store have proved its value. An attempt is made to maintain a stock of animals sufficiently large to care for the current needs of the school with some allowance for unexpected demands. Efficiency is achieved by the cooperation of department heads, who give adequate warning to the Animal House of unusual needs, and who, when possible, place in advance and by date their orders for animals used for teaching or for research projects. The manager of the Animal House has expert knowledge concerning sources of animals, the habits of dealers, care and feeding of animals and other data which he makes available to research workers in the school. The Animal House is open to inspection by any interested person who may visit without warning. The employment of responsible men of good character as permanent workers in the Animal House, the strict enforcement of the rule which permits the purchase of animals only from reputable dealers and the high standards of humane care given to all animals have served to win the approval of the anti-vivisection groups for our methods of handling animals, if not for the purposes to which we put them.

Housing of Animals

Animals may be housed in the animal room or quarters of a given department of the school or in one of two central animal houses which have been built for the purpose as structural additions to the school. Departmental animal rooms vary from simple laboratories converted for this purpose for temporary convenience to more elaborate and permanent units attached to certain departments of the medical school. Similar animal rooms and quarters are found in the teaching hospitals associated with the medical school. Of the two central animal houses, one, a recent acquisition, is designed particularly for the care of animals used in the study of infectious disease. Its basic plan is that of a cooperative apartment house with completely independent units cared for by the departments using the units. The second central animal house is the one which contains the retail store for the sale of animals. This has an additional function, that of a hotel, in the renting of cage space.

Entire rooms or portions of rooms may be leased to research workers who, themselves, provide food and care. If it is desired to have animals cared for and fed in the central animal house, a set charge is made which includes all care, food, overhead and labor. The charge for boarding animals is computed on a cost basis. At the present time, the following charges are made for boarding animals (including room, food, and care):

Table 1

Mice	20¢ per dozen per day
Rats	20¢ per dozen per day
Guinea Pigs	2¢ per day
Rabbits	5¢ per day
Monkeys	22¢–28¢ per day
Chickens	5¢ per day
Cats	15¢ per day
Dogs	40¢ per day

Cost of Animals

The charge for animals, made by the Animal House to the research worker, is based on the formula of cost, plus 10 per cent. This surcharge is omitted, in special cases, if no expense to the Animal House is entailed in the transaction. The amount of surcharge varies somewhat from time to time. It is so calculated that the books of the Animal House will show neither loss nor profit at the end of the year. The medical school furnishes heat, light, water, and the building. All other expenditures of the Animal House are defrayed by this surcharge or by the fee for the rental of rooms or cages in the central Animal House. Some variation in the cost of animals is occasioned by the loss of stock, either from deterioration in transit or from infectious disease after arrival in the Animal House. An effort is made to distribute the cost of such loss as evenly as possible, so that no one department will suffer unduly, if there is an epidemic with a high mortality rate among the animals used particularly by that department. Recently, approximately 20 per cent of the cats used in one year died shortly after arrival at the Animal House. The existence of a retail store in the Animal House permitted that loss to be absorbed without upsetting, to a serious degree, the budgets of these department or research workers using cats. The formula of cost, plus 10 per cent surcharge, which is now in effect, brings the total cost to the research worker of the various animals handled by the Animal House to the following levels:

TABLE 2

Mice	18¢ to 23¢
Rats	55¢ to 75¢
Guinea Pigs	85¢ to $1.25
Rabbits	35¢ per pound
Monkeys	$30.00 (subject to change)
Chickens	about $2.50
Cats	$ 1.50 to $3.00
Dogs	$ 3.50 to $6.00

Method of Paying for Animals

There are roughly three sources of funds which are used to pay for animals at Harvard Medical School: (1) the departmental budget, obtained from university funds and assigned by the Administration of the University; (2) special research grants, obtained either from funds entrusted to the University for special purposes, or from foundations interested in the support of research; and (3) the research worker himself. Payment by the individual is not a common practice and is encountered only when a clinician conducts occasional part-time research or performs diagnostic tests on animals for his own benefit. The amount of money obtained from the departmental budget, or from sources outside of the regularly assigned funds to one department, varies according to the department, the character of the projects being investigated, and the size of the project. In general, departmental budgets care for animals used for teaching purposes and for a limited number of experimental animals. Most of the large projects which make use of great numbers of animals over a long period of time are supported by funds not ordinarily provided for by the departmental budget.

University Breeding Farms

The Harvard Medical School conducts no breeding farm as a part of the Animal House. Individual departments may raise mice or rats or guinea pigs, if a research project necessitates the control of factors which cannot be controlled when the animals are procured from commercial dealers through the Animal House. A summarizing statement may be made that it has been found more advantageous to purchase from reputable dealers most of the animals used than to raise them on our premises. The reasons are many. We may mention the amount of space which would be required and the special equipment. Large stocks of mice, rats, guinea pigs, rabbits, etc., far beyond the actual number of animals used, would have to be kept on hand to meet the demands for animals of a specified age, weight, sex and breed and to offset the depredations caused by epidemics. To maintain such large colonies for the production of the animals needed, great expense would be incurred. This would have to be met in one of two ways: either the medical school breeding farm would have to seek markets outside of the medical school to take care of the surplus animals, and so become, in fact, a commercial breeder, or the cost would have to be absorbed by the medical school and individual departments. This would increase considerably the cost of research. These statements apply to animals used for most of the research work at the Harvard Medical School. Occasional projects, such as experiments concerned with certain aspects of cancer and genetics, demand the maintenance of large colonies of mice, but here the maintenance of the colonies is a part of the research project itself. When large numbers of mice of a specified age and rigidly controlled environmental conditions are required for infectious disease studies, it is necessary to maintain breeding colonies. At the present time, the surplus from one such colony is sold at the rate of 15¢ per mouse, a figure which, under favorable conditions, covers overhead, food, care, depreciation of equipment used, and, perhaps, a small profit. Occasional epidemics in such colonies may distort these calculations considerably and cause a loss which has to be borne by the department itself. The present practice of the Animal House is to raise no animals and to place reliance upon reputable commercial breeders. Of these, there is a sufficient number. Much of the responsibility for the research supply of some of the animals used in medical schools, as, for example, the rat or the mouse, has been lifted from medical schools by the existence of research institutions such as The Wistar Institute and the Jackson Memorial Laboratory. Such institutions, by their contributions to our knowledge of the biology of the rat and the mouse, and by the development of strains of these animals designed for specific purposes, have provided medical research with an invaluable background for the use of these animals in the solution of problems in human medicine.

The Dog and the Cat

All mammals required for teaching or for research, except the cat and the dog, may be procured without too much difficulty, under ordinary conditions, either from commercial breeding farms or from importers, as is the case with monkeys. No commercial breeder now produces dogs and cats for use in research or teaching. The situation of the medical schools in regard to the supply of dogs and cats is a serious one. In Boston, all cats and dogs are supplied either by a small number of licensed dealers in the vicinity of Boston, or by dealers in Pennsylvania, New York, Delaware and New Jersey. Animals are purchased only from licensed dealers who meet the requirements of local governmental agencies. There is no traffic with boys or with private individuals. It should be noted that all dogs and cats purchased have been discarded by their owners for one reason or another. Research workers, therefore, are compelled to accept, for their purposes, dogs and cats of uncertain age, of unknown genetic constitution and of variable past history, as far as health and treatment are concerned. Frequently, these animals are ill or must recover from the rigors of a long train or auto journey, which is a hardship even under the most humane conditions. The cost of cats varies from $1.50 to $3.00, and of dogs from $3.50 to $6.00 per animal. Included in this cost, is the expense of transportation. In Boston, approximately 1500 dogs and 2000 cats are used each year in the medical schools and research laboratories. In the same city, the animal leagues and humane agencies, acting as animal pounds for the city of Boston, destroy by electrocution approximately 35,000 dogs and 65,000 cats a year. In Chicago, St. Louis, Houston, Durham, No. Carolina, and one or two other cities in the country, dogs may be obtained for the purposes of teaching or research from the city pound under the provisions of a city ordinance. The cost to the medical schools and laboratories is merely that of handling the animals. It varies from $1.10 in Chicago to $1.63 in St. Louis. The cost to the city for the maintenance of the pound or the gathering of stray animals is not included in these figures. Approximately, 10,000 dogs per year are used by the medical schools of Chicago for scientific purposes.

The uncertainty of the supply of dogs and cats in most cities of the country, the great variation in the condition of the animals, and the lack of information concerning their genetic constitution and health background have been responsible for repeated attempts in several parts of the country to raise dogs and cats for experimental purposes. Some of these projects have been suggested by men who have had long experience in the raising of mice and rats, and who are disturbed by the reliance of medical workers on data obtained from the use of dogs and cats of such uncertain background. Estimates concerning the cost of raising a dog vary greatly in different parts of the country. For one attempt, it was learned that it cost about $15.00 to raise a puppy to the age of six months, a figure which does not include the cost of maintenance and services and the investment for buildings and equipment. Under university conditions in the south, the cost of feeding and caring for one dog for one month was found to be $3.00. This figure includes food, labor, and bedding, but not the overhead of equipment and maintenance. Nor does this figure indicate the cost of breeding animals and raising puppies to adult life, with all of the additional expenses implied by such a project and all of the loss which might be incurred as a consequence of infectious disease and epidemics. Estimates by different workers range from $25.00 to $75.00 as the cost of breeding and raising one dog to maturity. It is obvious that these estimates are not based upon the same data. The larger figure is probably nearer the cost, if buildings and equipment are included.

There are many workers in medicine, today, who favor the use of pedigreed dogs for research purposes, and believe that a great opportunity for research might be provided, as a by-product of such a project, by the availability for study of large numbers of dogs produced under controlled conditions from selected stock. It appears clear that the expense of raising such dogs for teaching and experimental purposes must exceed, to a very great extent, the cost of $3.00 to $6.00, when the animals are procured from licensed dealers, or the even lower cost, when the animals are obtained from the city pound, as in St. Louis and Chicago. No data are available concerning the large-scale breeding of cats for experimental purposes. A constant supply of dogs and cats, raised for the purpose, under conditions comparable to those which govern the raising of mice, rats, rabbits and guinea pigs, and free from the activities of anti-vivisectionists, represents a goal, the attainment of which is surely worthy of great effort. Some solution, not now apparent, must be discovered, however, to bring the cost of commercially bred dogs and cats within the reach of university budgets and foundation grants.

ANIMAL COLONY MAINTENANCE—FINANCING AND BUDGETING; VIEW-POINT OF THE COMMERCIAL BREEDER

By C. N. W. Cumming and F. G. Carnochan

Carworth Farms, Inc., New City, N. Y.

It is an old and time-honored statement that supply is regulated by demand and that, when a demand exists, sooner or later a supply will come into being. The business of raising laboratory animals came into existence to supply the demand for test animals which was caused by the increased activity of medical research, and the necessity of using animals to test the new biological agents coming into use. The rapid growth of experimental medicine since the last world war has encouraged the growth of a firm such as ours, whose entire activities center around the production of uniformly high quality laboratory animals.

In the dark ages of experimental medicine, any type of laboratory animal was difficult to obtain and many an experimenter was limited in his work because of this shortage. Two palliatives were resorted to in order to overcome this condition. Some of those who could command the necessary facilities, and who had sufficiently large budgets, started to raise their own animals. In this discussion, we will call them consumer breeders. There were others, who either did not wish to enlarge the scope of their activities to include animal husbandry, or who did not have the necessary funds available. These took the easier way of encouraging individuals in the vicinity, whom we shall call home breeders, to raise the particular type of animal that was required.

Consumer Breeder

Among the consumer breeders, were many pharmaceutical houses who kept accurate records of their costs. In most instances, after extensive experience, these costs proved excessive and the operations were discontinued. These high costs were, in large part, due to the fact that it was impossible to correlate production with demand. This resulted in an excess of animals, which had to be destroyed when they were not required, and in a shortage in times of great need. This type of consumer breeder, therefore, turned to the commercial breeder as a dependable source of animals bred to specific standards at a reasonable price. Consumer breeders that continue to raise animals, regardless of cost, are those institutions which produce only a small part of their requirements; those few which are endowed with unlimited budgets; and some commercial houses that are compelled to do so by government regulations.

Home Breeder

Those who had chosen the encouragement of home breeders, as a means of solving their problem, also found themselves in difficulties in so far as supply was concerned. This was due to unforeseen increases in their requirements, as well as to the difficulty of estimating future demands. The small breeder, on the other hand, who started to raise animals for some particular laboratory, soon found himself in trouble, partly, from his own inability to look on his newborn enterprise as a business, and partly, from the very uncertain demand for his animals. The home breeder is usually engaged in breeding as a means of added income, using waste space which is frequently inadequate and unsuitable for the purpose. An additional disadvantage is his lack of basic biological training necessary for the scientific breeding of specific types.

Many of these new breeders have started to raise animals with the high hope that they would not have any trouble marketing their product, but have found themselves left high and dry, in spite of an overall increase in demand. This condition has obtained because the small breeder has been dependent for his sales on one local laboratory or institution, in which the demand has, in accordance with the very nature or research work, varied considerably. If a research project, which necessitated the regular use of a large number of animals, is suddenly concluded, and no other work is started immediately, the local breeder may be without his market for a matter of months. After a few futile attempts to sell his animals elsewhere, he usually decides to go out of business. On the other hand, if he is lucky enough to find another laboratory to use his animals, the agony is only prolonged, since, sooner or later, that project ends and he again has no market for his animals. He then is faced with the necessity of either killing his stock or going bankrupt feeding them. Furthermore, he has no means of determining actual costs, since, by ignoring the hidden costs, he generally considers his profit as the difference between his sales receipts and his direct expenses. Eventually, he finds the return inadequate, and, with better opportunities in other directions, he gives up the venture. For these reasons, consumers have generally found that the home breeder is not a dependable source of standard animals and have turned to the large commercial breeder for their requirements.

Commercial Breeder

Because the commercial breeder must operate on an extensive scale, he must provide adequate capital for his needs. Also, the diversified nature of the management of such a business requires that several men, with capabilities to cope with the varied problems, join in the enterprise. It is clear, therefore, that the corporate structure is ideally suited to meet these conditions. This requires the organization of a corporation, with its attendant expense, as well as the sale of stock to qualified men for sufficient capital to do business.

While this capital investment meets the basic needs, an examination of the requirements of a well-run integrated business will show the need of additional outside capital. This may be secured by means of mortgages on real estate and chattels, bank loans and by indebtedness to creditors for purchases made.

These varied forms of invested capital will be evidenced by the following assets on the books of the company.

Cash. A sufficient balance of cash must be maintained in the bank account to meet the current needs of the business, such as weekly payroll, purchases of materials and supplies, and operating expenses, such as electric light and power, rent and telephone.

Accounts Receivable. Since sales are generally made on the basis of credit, and payments, therefore, are not received until the lapse of thirty days or longer, sufficient capital must be provided to carry such accounts until payment is received. This amount will be considerable as it may equal one-sixth of the annual sales.

Inventory. In order to have stock for sale, a breeder herd must be maintained. Since this herd is comprised of specially selected animals which must be kept for a considerable time in reserve before they become satisfactory replacements for dead or obsolete animals, the cost of such animals is determined to be equal to five times the cost of animals held for sale. In the case of mice, this breeding herd must be reconstitued on a seven- or eight-month basis depending on the strain. That is, no mouse is permitted to remain in the breeding colony for a longer period. Our experience has shown that the quality and quantity of the progeny beyond that point does not warrant the cost of maintenance.

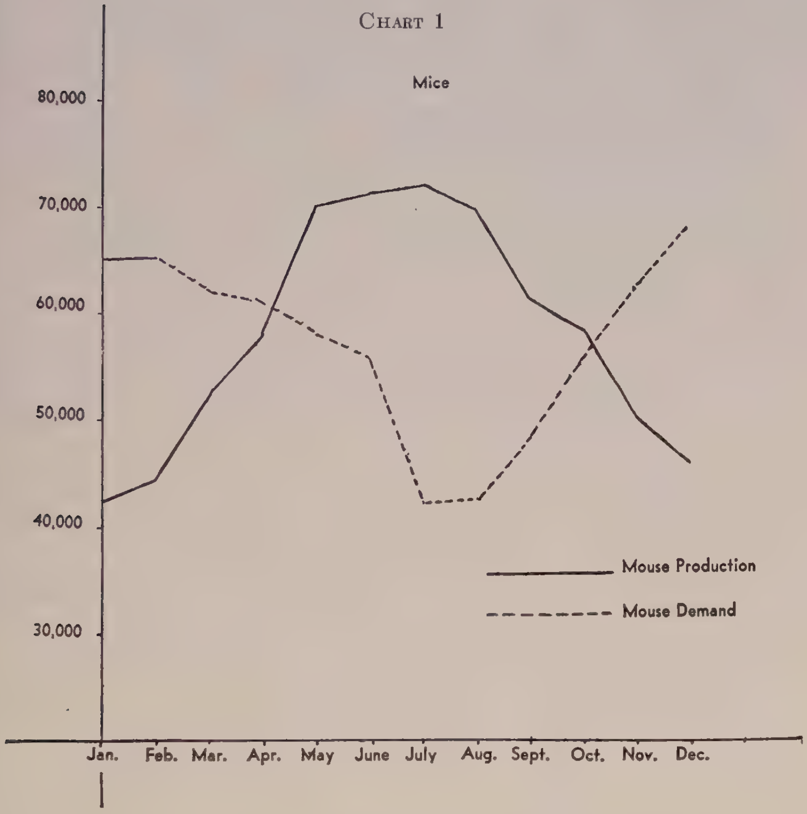
The size of the breeding herd is dependent upon the forecast of future need for animals. It is estimated that, to increase the sales stock of mice by fifty per cent for a period nine months hence, it is necessary that the breeding herd be increased ten per cent per month for five months. To increase the breeders by ten per cent, it is necessary to introduce a gross total of twelve per cent. The period of nine months, mentioned above, is the average time required to complete a program of expansion of any size, provided the sales stock is kept at a level sufficient to ensure income for necessary operations. In this way, a sufficient sales stock of animals of different sizes and strains is on hand, at all times, to supply, at least, the most urgent demands during expansion. Incidentally, the job of attempting a selection of the most urgent orders is a thankless one, because invariably every order is considered a rush order by the consumer, who deems it a personal affront if his requirements are not met at once. The demand for animals cannot be satisfied on such short notice as orders for manufactured goods, but must be met by production estimates long in advance of anticipated sales. An important factor to keep in mind in this connection is that, as a rule, the period of greatest demand does not coincide with the period of greatest production (CHART 1). It is, therefore, necessary to balance all factors in planning the production, since an oversupply results in losses in two directions. The cost of feeding and servicing the animals is lost if they are not sold, and the animals must be destroyed when their age makes them unsalable. Then, too, the overcrowding of the cages make the possibility of disease an ever-present danger.

With these factors in mind, our experience has shown that the inventory of mice breeders on hand should, at an average, equal approximately two-thirds times the anticipated weekly sales; and, for guinea pig breeders, six times the anticipated weekly sales.

CAPITAL EQUIPMENT. The business of raising animals as a commercial venture necessitates investment in many kinds of capital equipment. Most of these items are of a type peculiar to this business. The buildings should be erected specially for this purpose and should contain many essential features, such as air-conditioning, vermin control and disease barriers. They should be designed for efficient servicing, feeding and cleaning. The grounds must be spacious and provide sufficient arable soil to raise part of the required animal feed.

The equipment of the building is of two types—fixed and operating. The fixed types include heating, plumbing, sanitation, air conditioning and animal shelves. The operating equipment includes such articles

CHART 1



as animal cages, cleaning equipment, bottles and feeding wagons. The same furniture and office equipment, necessary for any other business enterprise, is required by the commercial breeder, which includes desks, typewriters, safes, calculating and adding machines.

AUTOMOTIVE EQUIPMENT. Trucks must be provided for transportation between the different colony units and for other internal hauling work. Trucks are also required for deliveries to local customers, to freight and express stations, and for the pickup of incoming supplies.

Besides the above mentioned items of capital investment, it is found that some capital is tied up in other miscellaneous assets such as security deposits, prepaid insurance, loans and advances to employees.

Price Determination

One of the most important functions of management is the determination of the price of the product, because, if it is too high, the product is unsalable, and, if too low, the result is a loss. Another important function is the constant effort to reduce costs so that the selling price can also be reduced.

In determining the selling price, all the factors of cost must be considered (CHART 2). The direct costs include feed and labor, operating supplies and delivery expense. The indirect costs include supervision, laboratory expense, depreciation of equipment and repairs. The administrative costs must also be added and include executive salaries, office salaries, telephone and telegraph, postage, printing and stationery, business and property taxes, insurance, and interest on borrowed capital.

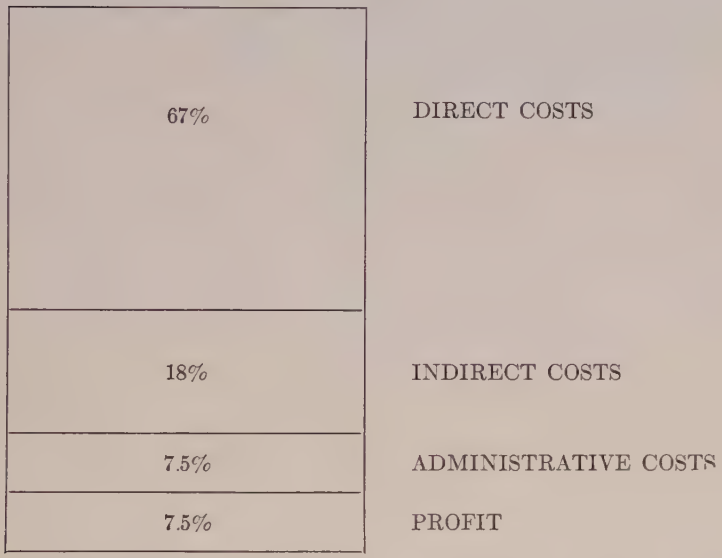
In arriving at the selling price, the total of these costs must be divided by the salable production, which gives the unit cost, to which must be added a profit. The factors which affect costs in this business are different from those met with in most businesses. A machine shop owner, spending a dollar in production, knows what his output will be within a very narrow margin. In this case, when a dollar is spent for production, the results can be estimated only on a widely fluctuating basis. The production for any period is not necessarily in direct proportion to the amount spent, but is affected by losses due to climatic fluctuations, disease, and other uncontrollable factors.

Many of the foregoing items of direct cost are apparent and are probably taken into consideration by all breeders. It is when we pass on the indirect costs that the major differences between apparent and real costs are encountered. It is the failure on the part of many breeders to take these indirect costs into account that encourages them to offer stock at lower prices, which results in the very high percentage of business failures in this industry.

Budgeting

Good management works on the basis of a budget prepared for the future on the experience of the past and providing for the probable demands. The budget must be based on the sales which are planned as probable, and the costs required to fill that demand. The costs must include all of the elements set forth below as aggregating the cost of operation. Provision for financing the budget must be planned so that the funds will be available when needed. If all the elements of the budget work out as anticipated, and efficient operation keeps the costs within the bounds set, then a profit on operations results. Failure

Chart 2



in any particular, or the occurrence of an unexpected calamity not covered by insurance, results in a loss.

The main elements of cost as planned in our budget are as follows:

A. Direct Cost (67 per cent)

1. *Feed:*

This item represents about one-third of our direct costs. Our practical research, comprising actual feed testing programs, has assured us of the best possible results for this important factor in animal husbandry.

2. *Direct Labor:*

This comprises about one-half of our direct costs, consisting of wages paid to animal tenders. All labor employed for this purpose is specially trained to perform every operation in the most efficient manner. These methods have been perfected as a result of detailed time studies.

3. *Delivery Costs:*

4. *Operating Supplies:*

Items three and four account for the remaining one-sixth of our direct costs. Delivery charges are determined by Railway Express Company rates, over which we have no control. Another item is the cost of shipping containers. These containers are used only once, due to the possibility of contamination from wild rodents en route. The budget for Operating Supplies is based on past experience and is kept as low as is compatible with efficient operation.

B. INDIRECT COSTS (18 per cent).

These include such accounts as supervision, laboratory expense, rent, electric light and power, operating expense, depreciation of buildings and equipment, repairs and indirect labor. Most of the above accounts are common to all businesses. However, the item of laboratory expense is not an operation that is commonly incurred in the laboratory animal breeding field. It includes the cost of testing the breeding herds for most of the common animal pathogens. Supervision is also noteworthy, for its covers a multitude of very necessary functions and controls besides that implied in the name. The packing and final inspection of all our animals is done by the supervisory force and, on the excellence of this work, to a large extent, depends our reputation. Also, under this heading, comes the keeping of all production records and the performance of genetic tests to maintain the purity and uniformity of our strains.

C. ADMINISTRATIVE COST (7.5 per cent).

This comprises the following expenses: executive salaries, office salaries, telephone and telegraph, postage, printing and stationery, legal and accounting, business and property taxes, social security taxes, insurance and interest on borrowed capital as well as miscellaneous expenses.

D. PROFITS (7.5 per cent).

Although our budget is planned for a profit of seven and one-half per cent of the selling price, our past experience has shown that unforeseen contingencies arise which may absorb a large part of this item. Furthermore, after making provision for expansion, it has been found that the capital invested has shown no return to the stockholders in the form of profits. The principals of the business draw a modest salary and are content in knowing that their contribution is a factor in the progress of medical research.

Discussion

The commercial breeder is able to command a wide and varied market for his animals. This is possible by reason of the fact that the company employs the services of at least two executive officers, one of whom looks after production and research, while the other confines himself to sales and supervision of the office. Both of these officers must coordinate their efforts to formulate the general policies which guide the company. At the same time, each must devote his energies to the departments under his guidance. In this respect, the commercial breeder has a definite advantage over the organization consisting of only one man, whose efforts are necessarily so divided as to cause detriment to either sales or production.

It is this ability to command a large market that enables a commercial breeder to produce large quantities of animals. This, in turn, makes it possible to maintain an inventory sufficient to supply, not only large regular orders, but also most emergency orders. The fact that the commercial breeder has proven his ability to stay in business, year after year, makes possible a constant source of supply. Because this supply is maintained, in so far as is possible, under standard conditions of environment and heredity, it enables the research worker to duplicate, not only his own experiments, but also those of other investigators who have used similar uniform strains.

The large scale of the commercial breeders' operations makes it possible to institute research programs, whereby strains are produced for specific purposes. The use of these specific strains makes it possible for the investigator to obtain satisfactory results with fewer animals, thus reducing the expense of his experiment, even though the unit cost of the animals may be slightly higher. Our company is eagerly awaiting the day when it will be possible to resume research dealing with animal diseases to which our own colonies may be susceptible. This research has been discontinued, due to the pressure of war orders, but plans for new investigations are well advanced, and it is anticipated that the near future will find them in progress.

The cost of animals is kept as low as is consistent with good business practice, and our company feels that a very necessary part of this cost is the maintenance of an efficient office staff. This enables correspondence to be dealt with promptly, and accurate records to be kept of all customer transactions, in addition to the usual business operations. We have found that the keeping of accurate sales information has enabled us to build up a fund of knowledge about our various strains which makes it possible for us to advise the research worker as to the type of animal most suitable for his particular problem.

The large commercial breeder of laboratory animals has been of material assistance to both government research programs and manufacturers of biologics during this war. His large production has gone a long way towards stabilizing his prices without any policing from the

O.P.A. During the last war, there was no organized business in this field, and, since the home breeder abandoned his colony to enter industry at a high wage, the shortage of mice was sufficient to drive the price to one dollar per mouse. During this conflict, the same condition applies, as far as the home breeder is concerned, but the large commercial breeder, since he expects to continue in business after the war, advances his prices only to the amount necessary to cover extra war costs. To our knowledge, the highest price charged for mice by a reputable breeder, during this emergency, has been 25¢ each.

Without overstatement, it may be said that the commercial breeder performs a vital function by supplying a suitable medium for testing medical theories and biological products.

ADDENDUM

Because of the great interest shown in the paper on heating, we thought this group might be interested in our experiences with heat controls.

In the first building which we erected, we installed a direct hot-air system with a blower behind it to force the air as evenly as possible to all parts of the building. This was controlled very simply by an electric thermostat which turned the oil burner off and on as heat was required. We have found many disadvantages in this system. The heat would fall very rapidly in certain sections before the thermostat could catch up with the temperature changes. The thermostat itself, because of the large amount of dust in the air of an animal house from the bedding and feed, became very irregular in its operations, and the fluctuation in temperature was very wide because of the stickiness of the contact in the thermostat.

In our second building, we adopted a much more elaborate system in which the air is blown over steam coils which have automatic valves pneumatically controlled. The thermostats which are set in the animal rooms are pneumatic also. The boiler is kept under steam pressure at all times, hence, responses to the thermostat are very quickly made and the temperature fluctuations are held within a one-degree range. The heated air, which is passed over the steam coils, is positively driven to each room by a powerful fan and there is equally positive exhaust from the room by a separate exhaust fan. As a safety measure, each room contains a thermostat, which pneumatically controls the current to the fans, to shut them off should the temperature exceed 80° in the room. There is always the possibility of a sticky steam valve, or a leak in the pneumatic system, which would permit the steam valves to open wide, and so overheat the room. Since this 80° thermostat shuts off the fan completely, no excess heat can be driven into the rooms in the event of such failure. In the air-return plenum chamber is a thermostat set for 60° which will also shut off the fans, in the event of a steam failure, and prevent cold outside air from being introduced into the rooms. This system has worked very well, but, since we believed it could be further improved, in our latest building, we have modified it by removing all the thermostats from the rooms and placing them in the return air current. The advantage gained by this is that attendants cannot shift the thermostat up or down. To date, the control of temperature in the rooms under the latter system has been even more accurate then under the former. The installation however, is so new that it has not yet had a chance to be tested under extreme weather conditions.

DISCUSSION OF THE FINANCING AND BUDGETING PAPERS

Dr. Ellis J. Robinson (*American Cyanamid Co., Stamford, Conn.*):

We have been breeding beagles and my figures agree with those given by Doctor Farber, of about 40¢ per day. My figures indicate that it costs about $150 to raise a pup to the age of a year. We have been using these dogs for a certain type of work and have found them to be a very worth-while experimental animal. I have heard opinions to the contrary about dogs.

Dr. Edmond J. Farris (*The Wistar Institute of Anatomy and Biology, Philadelphia, Pa.*):

I have heard that it is a question of the size of the animal and type of maintenance, that determines the variable costs in dog raising, with range from $15 to $45 per year.

Mr. Carnochan:

In 1938, we were asked to produce dogs in quantity for an institution that was working on immunization and distemper. The dogs would have cost $18 and, on that basis, they refused to buy, because they were too expensive.

Dr. Myron Gordon (*American Museum of Natural History, New York, N. Y.*):

There should be a clearing house for information from which we can obtain information on certain types of animals. The National Research Council should be able to establish such a clearing house where we can write and get the information we want. I should like to hear some comments on the subject and get some suggestions and have it brought to the attention of the National Research Council.

Dr. Clarence A. Mills (*University of Cincinnati, Cincinnati, O.*):

A problem in getting dogs for experimental purposes is that often it is difficult to obtain information concerning the genetic set-up of the dog. It takes time to get the dog in condition for the experiment. There should be a standard nutritional and metabolic basis for the raising of the animals for this purpose.

Dr. Robinson:

It is necessary to bring up the dogs and keep them for several months. Our experiments, done so far, have been of a hematological and histological type. The dogs have been most satisfactory. We have been using beagles and have used dogs of this kind that we know are completely healthy.

Dr. Mills:

Do you worm the dogs?

Dr. Robinson:

We have found a few worms on autopsy. We have maintained a very healthy condition for the dogs and examine them regularly. The problem should be brought to the National Research Council as to where one can obtain such dogs, with the assurance that they will be suitable for our purposes.

Dr. Herbert Clark (*Gorgas Memorial Laboratory, Panama*):

It is difficult to keep the dogs, parasite free. We have been able to produce good dogs even though they have been infected. The food is a very important item, as described here. We have been forced to use such things as rice. We let the dogs range during the day and lock them up at night. Our estimated cost per year is $40. We get native rice. The dogs produce well, and we do not worm them. Unless, of course, they have a severe case of worms.

Mr. Carnochan:

I should like to return to Doctor Gordon's remarks as to information, and where it can be gotten, about animals. I would like to see that section of the National Research Council, which is concerned with animal strains, establish a definition for "strain" so that we all talk about the same thing. At the present time, the word means anything from a haphazard colony which has been held a long time without outside admixture, to a group which has twenty-one or more generations of brother-sister mating behind it. Some trace back two or three generations before a common pair of ancestors can be found; some, ten, twelve, or even more. A scheme of inbreeding should be established, e.g., twenty generations of brother-sister, and from then on, first cousin, or double first cousin matings. These remarks apply to mice, but are equally true for guinea pigs, rabbits and rats. I suggest that no animal deserves the designation "strain," unless theoretically at least 80 per cent homozygous for all characters.

Dr. Gordon:

You must have a clarity of terms to get what you want. There must be some organization where these items can be straightened out so that we can get the animals we want.

Dr. Carl G. Hartman (*University of Illinois, Urbana, Ill.*):

The National Research Council is the logical place to establish such an information center.

Dr. Farris:

During the course of the second paper, question was raised how to increase the supply of animals during the winter season, when the demand was greatest and production lowest. My suggestion would be to modify the lighting conditions in animal colonies during the off-breeding season, to increase production. By simply increasing the length of day up to twelve hours by artificial lighting, there will be a forty per cent production increase.

Dr. Robinson:

About lighting, what do you consider a good proportion for good lighting with only artificial light?

Dr. Farris:

For rodents, 12 hours light and 12 hours dark to standardize the animals.

Dr. Gordon:

We have used light for increasing production in fish, and have increased production during the spawning season by photoproductivity.

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